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THE CONTROL OF CARBOHYDRATE METABOLISM[1]

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Submitted for publication November 9, 1933

LECTURE 1

A whole decade has passed since insulin became available for the treatment of diabetes and this has prompted me to devote the first two lectures to a review of the information which has emerged regarding its physiological action.

Its influence on carbohydrate metabolism, in company with that of other hormones, is profound, yet we must not lose sight of the fact that this intricate process is controlled by nervous mechanisms as well, and I shall devote the third lecture to a consideration of some recent experiments which have had as their object the elucidation of the nature of this control.

In discussing the mechanism of the action of insulin it will, I think, be most satisfactory if we revert for a moment to the experiments of Banting and Best, which gave convincing evidence that insulin could be obtained from extracts of pancreas. These experiments, you will recall, consisted in observing the effects on depancreatised dogs of injecting extracts which had been prepared by macerating in physiological saline solution the residue of pancreas which remains some weeks after ligation of the ducts. This procedure was adopted since it had been previously shown by several workers that such pancreatic residues still retain, more or less intact, the cells of the islets of Langerhans, while the acinar cells are so far degenerated that they have lost the power to produce zymogen granules, the precursors of the digestive enzymes. It was hoped that the hormone controlling carbohydrate metabolism and believed to be secreted by the islet cells would escape destruction by the enzymes. The blood-sugar level

[1] The Herter Lectures, delivered under the Christian A. Herter Foundation at the Johns Hopkins University, January 23, 25, and 27, 1933.

was taken as a guide, but it can scarcely be said that the results of the first few injections of extract gave any indication of the presence of the antidiabetic hormone, and it was only after repeated trials with increasing doses of extract, that definite lowering of the level was obtained. It was also noticed, however, that following the injections the amount of sugar excreted with the urine became decidedly lessened, and it was considered that the two results taken together justified the conclusion that the extracts contained insulin.

The problem now became one of finding some method by which the hormone could be extracted from normal pancreas for there was no hope of obtaining, by the duct ligation method, a supply of extract sufficient for extended investigation. Some method had to be found for extracting the hormone from pancreas obtainable in the slaughter-house and, as a first step towards this, the gland of the bovine foetus was used. It was hoped that simple saline extracts of such glands would retain their insulin, since it had been stated that no active digestive enzymes were present in them. Although encouraging results were obtained, the method was soon abandoned in favour of one in which the pancreas of adult animals (oxen) was extracted by alcohol, a watery solution of the resulting extract being used for injection. Under such conditions, the digestive enzymes are incapable of acting on the insulin which, being soluble in weak alcohol, becomes extracted. By using such extracts, Banting and Best were able to continue their injection experiments on depancreatised dogs and, as the number of results increased, it became more and more evident that the antidiabetic hormone was present in the extracts. It was an anxious time for all who were connected with the investigation. We knew that other workers had concluded that administration of pancreatic extracts prepared by somewhat similar methods could alleviate certain of the symptoms in both the experimental and the clinical forms of diabetes, and we hesitated to make claims to any further advance until it was certain that every source of error had been eliminated.

Although the antidiabetic action of the extracts, as judged by their effects on the blood-sugar level and the glycosuria, had not been striking, it had been consistently observed and this more than anything else convinced us that we were on the right path. Two prob-

lems required further study: the one, how to obtain extracts of greater potency and purity, and the other, the changes produced by the extract on other diabetic symptoms besides hyperglycaemia and glycosuria, especially the behaviour of glycogen and of the respiratory exchange in both normal and diabetic animals. The purification of the extracts was entrusted to J. B. Collip, and it is scarcely necessary for me to recall here the remarkable success which quickly attended this work and which led to the preparation of extracts sufficiently free from irritating impurities that they could be used for subcutaneous injection on patients. What interests us more particularly at present concerns the physiological effects of the injections. You will recall that in depancreatised dogs glycogen practically disappears from the liver and the respiratory quotient remains unchanged, even when the animals are fed with large quantities of food rich in carbohydrates. It was soon found that when insulin—as the pancreatic extract now came to be called—was also given, the liver became heavily loaded with glycogen and the respiratory quotient became noticeably raised, results which showed without any doubt that the hormone was necessary for the performance of the functions upon which both these changes depend. At the same time it was also observed that insulin diminished the excretion of ketone bodies in diabetic animals and that these improved greatly in health and lived much longer than when no insulin was given.

It seemed clear from these results that the fall in the blood sugar level following insulin must be due partly to conversion of the sugar into glycogen and partly to an increase in the rate of its oxidation in the tissues. Meanwhile, it had been observed that insulin also lowers the blood-sugar level, when injected into normal animals, and it was natural to suppose that the cause for this result must be the same as in diabetic animals: increased glycogen formation and more rapid oxidation of carbohydrate. Much to our surprise, however, this was found not to be the case. McCormick and I (46) injected large amounts of insulin of proven potency into previously fasted rabbits sometimes with and sometimes without sugar, but could not detect, by comparison with control rabbits not given insulin, any very definite effect on glycogen formation. Dudley and Marrian (25) at the same time found that the abundant liver glycogen of rabbits

fed on starchy foods was greatly reduced in amount when insulin was injected, while the muscles might become glycogen free. In the light of these experiments, it became evident that our problem was not so simple as at first sight it appeared to be, and although ten years of active research have passed, it cannot be said that we have formed any very clear idea as to how insulin affects glycogen formation.

TABLE I

Effect of insulin on glycogen

AUTHOR	ANIMAL	CHANGE IN GLYCOGEN	
		Liver	Muscles
Best, Hoet & Marks (10)	Cat	−	+
McCormick & Macleod (46)	Rabbit (fasted)	+ or −	+ or −
Dudley & Marrian (25)	Rabbit (fed)	−	−
Collazo, Händel & Rubino (15)	Guinea pig (fasted, then given sugar)	+	+
Cori (16)	Rabbit (fasted)	No change in 1 hr. then −	
Cori (17)	Mice	−	
Frank, Nothmann & Hartmann (27)	Rabbit (fasted)	+ (small dose)	
Barbour, Chaikoff, Macleod & Orr (5)	Rat (fasted)	− then + (small dose)	−
		− (large dose)	−
	Rat (fed)	− (all doses)	+ (large dose)*
Goldblatt (31, 32)	Young rabbit (fasted)	+	−
Corkhill (19)	Young rabbit (fasted)	+	−†
	Chicken	−	+
	Young ferret	No change	+
	Mice	−	+ (?)
Cori (18)	Rat	−	+

* L and M balanced.

† Loss in M greater than gain in L.

Many researches have been undertaken but the results have, in general, been confusing and contradictory. This is particularly so with regard to the liver, some authors claiming that insulin increases the formation of glycogen, while others assert that it has the opposite effect. Nor is the verdict concerning the behaviour of muscle glycogen by any means unanimous, for although, as Best, Hoet and Marks (10) showed, glycogen formation is always markedly stimulated when

insulin is added to the blood of the eviscerated spinal preparation, we could not demonstrate that this occurred in normal rats injected with insulin unless the dosage was excessive and the animals were given large amounts of sugar (Barbour, Chaikoff, Macleod and Orr (5)). In order to illustrate the inconclusive nature of the results in this connection, I have prepared a table (table I) in which glycogen formation is indicated by a *plus* sign and its diminution by a *minus* one.

This table does not by any means include all the investigations which are on record, but it gives a fair sample of the results, showing clearly that there must be some factor, unknown at present, which affects glycogen formation independently of insulin, and that the whole problem awaits further investigation. Insulin, no doubt, exercises some sort of control over the glycogenic function, but there are other influences as well which may entirely mask what this hormone does.

Nor can it be said that anything definite is known of the effects of insulin on the respiratory exchange of normal animals. In the earliest experiments of this type performed by Eadie, Dickson, Pember and myself (21) on dogs, it is true that a striking increase was observed in the consumption of oxygen and a less constant one in the respiratory quotient, but these changes bore no evident relationship to the fall in the blood-sugar level and may well have been dependent on the neuro-muscular hyperexcitability which becomes developed as the blood-sugar falls to low levels. Moreover, changes of the same type were not observed with rabbits and, as noted by Dudley and Trevan (24), a marked decrease rather than an increase in oxygen consumption invariably occurred when smaller animals, such as mice, were used. It would serve no useful purpose if I were to review the numerous observations on this aspect of our problem which are on record by various authors. The results are just as confusing as those pertaining to glycogen formation, although they seemed to show at least that the initial fall in the blood-sugar level, which invariably follows the injection of insulin, could not be accounted for by removal of sugar from the blood to form glycogen or to be oxidised. It was considered reasonable to suppose that the sugar disappeared because it was converted into some substance of unknown structure inter-

mediate between glycogen and glucose and which might or might not immediately affect glycogen formation or influence the rate of oxidation of carbohydrates. But it was impossible to identify any such substance and the possibility of its formation was thought by other workers to be ruled out by the results obtained by methods which were considered to be more strictly quantitative in character than ours had been. I should like to say something about these experiments here.

The first of them were performed by the late E. J. Lesser (41) and his pupils. Mice were used so that the entire animal might be subjected to analysis. Following injection of insulin more sugar disappeared from the body than from control mice not given insulin, the reduction being relatively greater for glucose than for glycogen. Since the amount of glucose lost, 34 mg., was insufficient to cause a measurable change in the respiratory quotient, it was impossible to determine whether increased combustion was responsible for the loss. The experiments were repeated with the difference that all the mice received sugar and it was now found that it became possible to demonstrate that, in those also injected with insulin, increased oxidation of glucose accounted for all the excess of sugar which was lost and which could not be accounted for by new glycogen formation. Reports then appeared of the important researches of Dale and his school in London, and of Cori and Cori in Buffalo. I cannot here discuss all of these results in detail, but there are certain of them which have so direct a bearing on our problem that I am compelled to devote some time to them, especially since they are widely accepted as proving that changes in glycogen formation and carbohydrate oxidation adequately account for all of the glucose which disappears when excess of insulin is present in the body.

After a long series of carefully conducted experiments, in which the effects of adrenaline, as well as of insulin, were studied, both on the fasting animal (rat) and during or following the absorption of sugar from the alimentary canal, Cori (17) sums up his results for the last-mentioned group, which may be taken as most nearly representative, in the following words:

> In the insulinized animals the agreement between the total amount of carbohydrate which disappears and the carbohydrate oxidized is fairly satisfactory.

It will be noted that in these post-absorptive animals with high glycogen stores an excess of insulin doubled the rate of carbohydrate oxidation. This increased oxidation occurred mainly at the expense of liver glycogen which disappeared nearly three times more rapidly than in the control animals. In contrast to this, muscle glycogen was not drawn upon to a much larger extent than in the control animals. There is of course the possibility that insulin accelerated the oxidation of muscle glycogen and at the same time increased the rate of glycogen formation from blood sugar in muscle. The other alternative is that blood sugar did not pass through a glycogen stage but was oxidised directly in muscle. The end effect is the same, *i.e.*, liver glycogen is called upon to replenish the blood sugar which is withdrawn in increased amounts by the muscle.

Essentially different in nature were the experiments of the London workers, and it is to these that I would more particularly call your attention since they bear, not only on the mechanism of the action of insulin, but also on certain fundamental principles of carbohydrate metabolism. They were performed on cats in which the spinal cord had been cut high up and the abdominal viscera removed with the exception of the liver. The vessels entering at the hilus of this organ were tied but, of course, the hepatic veins were left intact. A preparation consisting essentially of heart, lungs and extremities was thus obtained, and in the blood which circulated through it the percentage of sugar was found to fall, although no change occurred in the average percentage of glycogen in four corresponding groups of muscles dissected carefully at intervals from the muscles of the two hind-legs. When glucose alone was added to the blood by intravenous injection, it failed to bring about any formation of glycogen in the muscles or to cause an increase in the combustion of carbohydrates as judged by analysis of the air used to ventilate the lungs of the preparation. On the other hand, when insulin was injected along with the glucose, there was both formation of glycogen and increased combustion of carbohydrate. Encouraged by these results, it became important to see whether a balance could be struck between the amount of sugar which disappeared and that which could be accounted for by the extra glycogen formed and the increased combustion. The several items belonging to the credit and the debit sides of these accounts were fully tabulated and the final balances found to be so close that the authors felt justified in concluding that in the spinal eviscerated preparation, at least, all the sugar disappearing under the influence

of insulin is accounted for by increased combustion and glycogen formation. It was also pointed out, however, that the action of insulin in intact animals, both normal and diabetic, must involve some other function of carbohydrate metabolism besides these two, possibly an inhibition of the process of gluconeogenesis.

I find it difficult to understand why there should be this difference between the intact and the eviscerated animal while both

TABLE II

Just before insulin:		At end:	
Average Liver Glycogen	= 1.26 per cent	Average Liver Glycogen	= 0.70 per cent
Average Free Sugar	= 2.35 per cent	Average Free Sugar	= 1.30 per cent
Average Carbohydrate	= 3.61 per cent	Average Carbohydrate	= 2.00 per cent
	Difference 1.61 per cent		

Loss of Carbohydrate from Liver = 1.61×0.58 = 0.934 g.

Average Muscle Glucose before Insulin	= 0.25 per cent
Average Muscle Glucose at end	= 0.19 per cent
Loss	= 0.06 per cent

Total Loss of Glucose from Muscle	$= 0.06 \times 16 =$ 0.960 g.
Blood Sugar Fall from 0.240 to 0.130 per cent	= 0.110 per cent
Loss of Glucose from Blood	$= 0.110 \times 2 =$ 0.220 g.
Total Glucose infused	3.250 g.
Calculated Total Loss of Glucose	5.364 g.

Average Muscle Glycogen before Insulin	= 0.654 per cent
Average Muscle Glycogen at end	= 0.830 per cent
" Gain	= 0.176 per cent
Total Calculated Gain of Muscle Glycogen	$= 0.176 \times 16 =$ 2.82 g.
Oxygen used = 2.230 cc. at N.T.P.	
Glucose Equivalent of Oxygen used	$= 2.23 \times 4/3 =$ 2.97 g.
Total Glucose accounted for	= 5.79 g.

react to insulin in the same way. If increased glycogen formation and increased carbohydrate combustion suffice to account for all the disappearing glucose in the one case, why should they not also be alone responsible for all that disappears in the other. It has always seemed to me that certain of the items of the accounts must be inaccurate, the *plus* errors of some being effaced by *minus* errors in others, so that the balance is more apparent than real. Several

years ago, I tried to point out where I think these errors arise (42) and I do not propose to repeat a close audit of the accounts to-day. I do wish, however, to call attention to certain possible sources of error, since they involve fundamental principles in the methods of investigation of carbohydrate metabolism.

First of all, let us examine for a moment one of the balance sheets (table II). It will be observed that a total of 5.364 gm. glucose is considered to have disappeared during the insulin period and that this is made up partly of sugar lost from the liver and muscles and partly of the sugar actually injected, after making allowance for changes meanwhile occurring in the percentage of sugar in the blood and tissue fluids. We may call this the credit side of the account and, of the total, more than one-third is put down to carbohydrate coming from the liver and muscles. But it may be asked, how can the liver act in this way when the circulation of blood through it is stopped by ligation of the entering vessels? The authors believe that this is made possible by the patulous condition of the hepatic veins, thus allowing an ebb and flow, as it were, of blood between them and the vena cava. Any change in the carbohydrate content of a portion of the liver is taken to be proportional to the amount of glucose which might enter the systemic circulation by this pathway. But, granted that sugar under these circumstances may pass from the liver to the systemic blood—and my associate, Dr. Peterson (48), has observed that it does—is it likely that the amount discharged from one part of this large organ filled with practically stagnant blood will be equal to that from all its other parts? Unless it can be shown to be so, it is not permissible to compute the total discharge from changes in the percentage of glycogen and sugar estimated in pieces of the organ removed at intervals from different parts. Then there is much doubt as to the dependability of the chemical methods used for determining the free sugar of organs and tissues and I need scarcely point out that any error of this type will be greatly magnified when, as in these observations, the factor used for converting percentage differences into total amounts is a very large one.

But it is more particularly with the items on the debit side of the account that I wish here to deal. There are two of these, glycogen formed and glucose oxidised, each contributing about equally to the

total loss in the experiment we are considering. For the present, let us examine the basis of computation of the glucose oxidised. It depends on the amount of oxygen used from which, assuming that the respiratory quotient is unity, the carbohydrate oxidised is calculated on the basis that 750 cc. of oxygen correspond to 1 gm. of glucose. As a matter of fact, this was the quotient actually observed both before and after administration of insulin, which would seem to afford ample justification for its use in the calculations. This means that, even before insulin was given, the only fuel being oxidised by the eviscerated preparation was carbohydrate; in other words, that muscle by itself has this quotient, for muscle is the only active tissue remaining in the eviscerated preparation, the heart and lungs merely functioning as pump and aerator for the blood which circulates through the muscles. At the time of their publication these results on the eviscerate cat seemed to confirm those obtained by Meyerhof (47) for the respiratory quotient of isolated frog muscle and to be substantiated by the conclusion of A. V. Hill, Long, Lupton and Furusawa (29) that the respiratory quotient of muscular exercise in man is also unity when measured for a period of sufficient length so that the oxygen debt incurred during its actual performance had been completely repaid.

We must pause a moment to consider the implications of these conclusions for there are few fundamental principles of metabolism of greater importance. If it be the case that the respiratory processes in muscle involve only carbohydrate, then we are driven to the further conclusion that protein and fat must be converted into it before being used for the production of muscular energy.

Quite apart from their importance in connection with the problem of the mechanism of the action of insulin, it was evident, therefore, that the experiments on eviscerated animals should be repeated in order to see whether some factor other than the processes of muscular metabolism might be responsible for the high quotients. Changes in the combining power of the blood and tissues for carbon dioxide is the most likely disturbing factor in this connection so that I suggested to Dr. Kilborn (37) that he should repeat the evisceration experiments and determine, not only the respiratory quotient, but also the CO_2 percentage of the blood at intervals both before and after the opera-

tion. As Burn and Dale (12) had stated, Kilborn (37) found that the quotient rose immediately the viscera were removed, but he also observed that the blood CO_2 fell and, when the observations were continued for some time, that the respiratory quotient might rise even to well over unity. This made it almost certain that increased excretion of CO_2 from the blood, at least in part, was responsible for the high quotients. It was subsequently shown that this excessive loss of CO_2 was due partly to overventilation of the lungs after re-

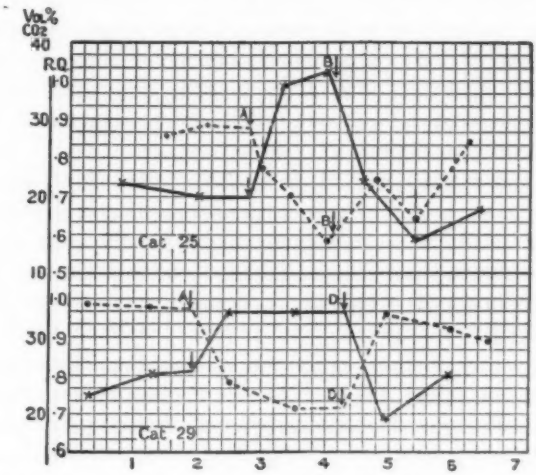

FIG. 1. The interrupted lines represent CO_2 in blood (volumes per cent.), and the continuous lines represent the respiratory quotient. *A* = eviscerated. *B* = lessened ventilation. *D* = breathing expired air. Time in hours.

moval of the viscera, thus causing a blowing-off of CO_2 and partly to the accumulation of lactic acid in the blood, causing a splitting-off of CO_2 from bicarbonates. When Kilborn retarded the rate of excretion of CO_2, either by closing down the rate of the respiratory pump or by adding 2 per cent CO_2 to the inspired air, quotients of less than unity were obtained, as shown in the curves of figure 1. When the amount of CO_2 that was inspired in excess of that produced by metabolism was calculated, it was found greatly to exceed the amount that could have come from the bicarbonates of the blood

and tissue fluids alone. This could only mean that the solid tissues were also contributing some of the excess CO_2, and Laurence Irving (36) and his co-workers in Toronto have published several papers bearing on this aspect of the question. It is one of fundamental importance in connection with the acid-base equilibrium of the organism and does not directly concern us here. The last published paper of the series, however, does directly concern us, for it gives the results of some experiments in which the respiratory quotient did not become materially changed after evisceration, this being achieved by taking great precautions to adjust the artificial respiration to the needs of the preparation for oxygen. Kilborn's (37) and Irving's (36) researches show then that the respiratory quotient in the spinal preparation does not necessarily become raised to unity by removal of the viscera.

On account of these difficulties in the adjustment of the artificial respiration to the oxygen requirements of the eviscerated spinal preparation, and because of the importance of knowing the exact respiratory quotient of mammalian muscle, it seemed worth while to repeat the experiments in cats in which the brain stem was cut above the level of the respiratory centre so that natural breathing might not be interfered with. In this decerebrate preparation, breathing would become automatically adjusted to the respiratory requirements so that there could be no danger of blowing-off of CO_2 by over-ventilation of the lungs. I suggested to Dr. J. M. Peterson that he should join in the solution of the problem by undertaking experiments of this type, and I wish now to give you a brief account of the clear cut and convincing results which he and I. A. Anderson and R. A. Cleghorn (3) have obtained. After decerebration under ether anaesthesia, the cat was left undisturbed for a few hours, during which its temperature was closely watched—any changes being guarded against by adjusting the heat of the table—and samples of blood were removed for estimation of the percentage amounts of sugar, lactic acid and carbon dioxide. The preparation breathed spontaneously and, at intervals, the expired air was collected for determination of the respiratory exchange. After the temporary disturbance due to anaesthesia, these various values as well as the blood-sugar percentages, were found to remain unchanged for many hours. This indicates

that the processes of metabolism were going on at a steady rate, thus providing us with a suitable preparation on which to investigate how these may be influenced by various factors, such as evisceration. It is necessary to point out that the level of decerebration in all these experiments was anterior to the pons, for we shall see in a subsequent lecture that when this region is involved, a prolonged hyperglycaemia and other changes occur.

The arterial blood pressure maintained a level not greatly below that of the intact animal, a fact which I consider of very great importance, since it means that the blood circulation through the muscles can have been little disturbed. In this regard the decerebrate preparation is decidedly superior to the spinal one, in which it is usual to find much lower pressures. It is of practical importance to note, however, that the removal of even a few cubic centimetres of blood may cause the blood pressure to fall and, to avoid this, it was necessary to adopt for blood analyses, methods which required only the smallest volumes of blood consistent with accuracy.

The results which concern us at present have to do with the behaviour of the respiratory quotient, the O_2-consumption and the percentages of CO_2 and lactic acid in the blood. The respiratory quotient immediately following evisceration was never anything like unity except when the rate of CO_2-loss was exceptional. It was usually higher than before evisceration and the average value for some hours in a number of experiments in which the rate of blood CO_2-loss was small was about 0.8, the value before being 0.77. This is perhaps the most important of all the results, for it shows not only that the factor used by Best, Dale, Hoet and Marks (9) for calculating the carbohydrate oxidised by the eviscerate preparation was incorrect, but also that the fuel of muscular activity is not exclusively preformed carbohydrate but that protein and probably fat must also be used. It is, of course, possible that these two foodstuffs are converted into carbohydrate locally in the muscle before they are used as fuel, and since this process of gluconeogenesis would require oxygen, quotients of less than unity might be explained. But this is a useless hypothesis since it cannot be put to the test of experiment, with the biochemical methods at present available. The conclusion that the respiratory quotient of muscle is less than unity is in harmony with

that of Doisy (22), Himwich (34) and others, who measured it by analysis of the gases in the arterial and venous blood supply.

It remains for us to explain why the respiratory quotient often does rise to unity in experiments of this type. In some of them, as

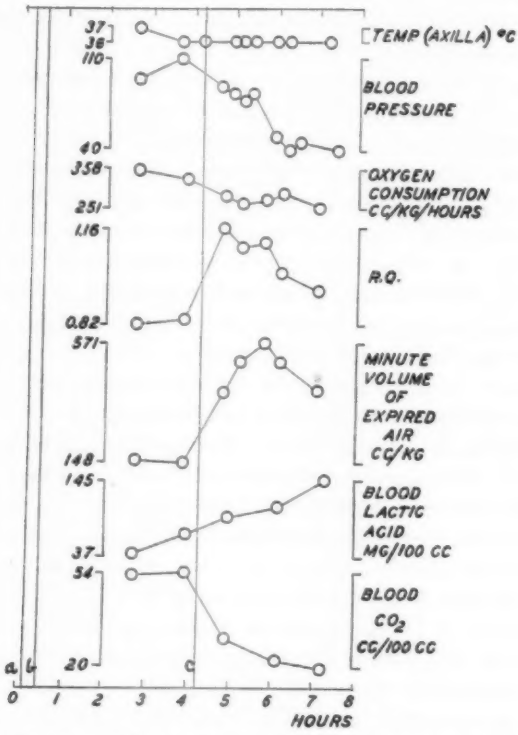

FIG. 2. *a*. Ether anaesthesia begun. *b*. Decerebration. *c*. Abdominal evisceration.

is shown in the curves of figure 2, this may occur almost immediately after evisceration; in others it occurs later. What is the cause for this difference? The clue is obtained by examining the behaviour of the percentages of CO_2 and lactic acid in the blood; the former fell rapidly and the latter rose to a corresponding degree when high

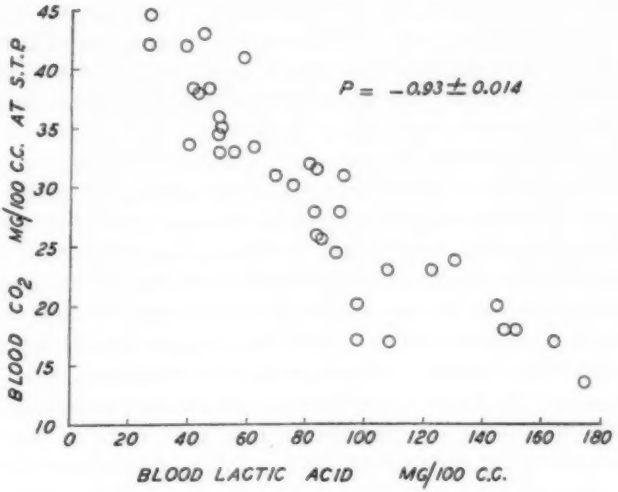
P = −0.93 ± 0.014
BLOOD CO_2 MG/100 C.C. AT S.T.P
BLOOD LACTIC ACID MG/100 C.C.
45
40
35
30
25
20
15
10
0
20
40
60
80
100
120
140
160
180

FIG. 3

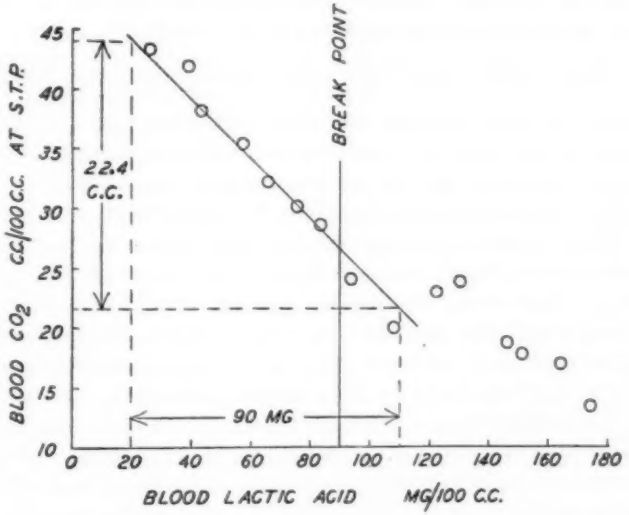
BREAK POINT
22.4 C.C.
90 MG
BLOOD CO_2 CC/100 C.C. AT S.T.P
BLOOD LACTIC ACID MG/100 C.C.
45
40
35
30
25
20
15
10
0
20
40
60
80
100
120
140
160
180

FIG. 4

quotients were obtained but these changes were much less marked when the quotient was low. There were also other striking differences between the two groups of experiments, the volume of respired air rising rapidly and the O_2-consumption falling only slightly in those with rising quotients.

A similar relationship between lactic acid and CO_2 was also shown by Kilborn, Soskin and Thomas (38) using the spinal animal and, although it perhaps leads us a little off the main path, I should like to discuss briefly its significance. In figure 3 are plotted the points representing the values for CO_2 and lactic acid concentrations simultaneously obtained in six experiments which were flawless from a technical standpoint and in which the changes we are discussing occurred sooner or later. There can be no doubt that a close correlation exists. In figure 4 Dr. Peterson (48) has grouped these points in subranges of small magnitude (10 mg. lactic acid and 5 cc. CO_2 per 100 cc. blood) and plotted the mean of each subrange, with the result that the very important fact is revealed that these points, at least up until what is styled the "break-point" is reached, fall very close to the line which expresses the relationship between the quantities of lactic acid and CO_2 involved in the equation

$$NaHCO_3 + CH_3 \cdot CHOH \cdot COOH \cdot \rightarrow CH_3 \cdot CHOH \cdot COONa + CO_2 + H_2O$$

This close fit must indicate that lactic acid production is alone responsible for the excess of CO_2 given out by the decerebrate, eviscerate preparation over the amount actually produced by metabolism, and since the respiratory quotient, up to the break-point, does not at least rise to any great extent we must conclude that when the reaction proceeds slowly, it does not produce CO_2 quickly enough to raise the quotient. Beyond the break-point, however, the CO_2 of the blood falls rapidly and the quotient rises. In the spinal eviscerate preparation, we saw that overventilation of the lungs by the respiratory pump was also responsible for the excessive quotients, but it is obvious that overventilation from this cause cannot come into play in the decerebrate animal, because breathing is under the control of a nerve centre, whose activities are mainly dependent upon the metabolic activities of the tissues. The magnitude of the respiratory quotient will accordingly depend on the rate of production of lactic acid and

when this is very rapid, as in the results shown in figure 2, quotients approaching or even exceeding unity may be obtained.

These results show conclusively, I think, that the respiratory exchange of the spinal eviscerate preparation cannot be taken as a reliable basis for calculation of its respiratory metabolism unless, as Irving, and others (36) have shown, very great precautions are taken to regulate the artificial respiration, and that it is permissible to use the decerebrate preparation for this purpose provided only that it is not producing lactic acid at an abnormal rate. When the rate of production of lactic acid in a good preparation is occurring slowly, the error in the respiratory quotient is insignificant until about 80 mg. per 100 cc. of blood is present, but beyond this the rate of production becomes accelerated, the CO_2 percentage falls rapidly and the respiratory quotient mounts to abnormal heights.

This, as I remarked, is designated the "break-point" and, when it occurs early, the preparation is useless for studies in metabolism. I should have liked to discuss with you the factors determining the rate of this lactic acid production but time will not permit.

I hope that you will agree with me that this considerable digression from the straight path of carbohydrate physiology is justified by the light it throws on the sources of error which make it impossible to calculate from the respiratory exchange of the eviscerate preparation the amount of carbohydrate which is undergoing combustion.

It is for these and the other reasons which I have given, that I am not willing to accept as correct the balance sheet submitted by Dale, Best, Hoet and Marks (9) for the carbohydrate metabolism of the spinal eviscerated cat. The results obtained on intact animals by Lesser and his co-workers and by the Coris do certainly show that relatively more carbohydrate is oxidised and relatively less glycogen formed after injecting insulin than when physiological amounts of this hormone are alone present in the organism, but this is only the final outcome of its action and does not necessarily indicate the nature of the first steps upon which must depend the sudden fall in the blood-sugar level.

The experiments which I have discussed in this lecture leave us therefore pretty much where we were at the start. Although they have been conducted with the greatest care, the results have only

served to show that there are fundamental details of technique which must be worked out before we can expect to make further progress. At the present stage, I believe that these concern especially the determination of glycogen in the liver and tissues, and I shall devote the first portion of my next lecture to a discussion of this problem.

LECTURE II

In my last lecture I presented evidence to show that we cannot as yet explain the mechanism by which insulin acts, and that it is unlikely that we shall be able to do so until more is known of the conditions which determine glycogen formation and carbohydrate oxidation in the animal body. What appeared at first to be a simple explanation of its action in diabetic animals—increased glycogen formation and carbohydrate combustion—proved to be inapplicable in accounting for the immediate fall in blood-sugar percentage which follows insulin injection in normal ones. It is obvious, therefore, that we must go back to fundamentals and investigate in closer detail the conditions under which glycogen is formed and broken down in normal animals, and the relationship of these functions to changes in the respiratory exchange. In to-day's lecture I wish first of all to discuss the conditions, apart from insulin, which have an influence on glycogen formation and to indicate where other, at present unappreciated, influences should be investigated. I will then add a few words to what I have already said concerning the unreliability of the behaviour of the respiratory quotient as a criterion of the extent to which carbohydrate oxidation is occurring. With regard both to glycogen formation and the respiratory quotient, I think you will agree, when we have considered the pitfalls, that many of the recorded researches on the effect of insulin on these functions are of questionable value. In the hope, however, that it may give us some guidance for future work, I wish to call your attention briefly to certain of the experiments, the results of which are summarised in table 1 and in which, arbitrarily perhaps, the conditions were more or less adequately standardised.

Following its discovery in 1857 by Claude Bernard (8), reports dealing with the amount of glycogen in various tissues in different species of animals were frequently published, but few of the results

are of much value nowadays. This is owing partly to the use of faulty chemical methods and partly to a failure to see that the animals were really comparable in regard to their treatment before death and that the tissues were removed with adequate precautions against post-mortem change. A great advance was made, especially in connection with the methods of analysis, when in 1901 Pflüger published a long series of researches and gave us the excellent method for glycogen determination which bears his name. Incidentally, I am not certain that the glycogen which is precipitated by alcohol in his method is actually the same as that present in the tissues, since heating in the presence of strong caustic alkali may split off some group normally present in the glycogen or bring about some change in its molecular structure. But the method has proved to be most useful and it is of great accuracy from the analytical standpoint. Where I consider insufficient care has been taken is in the selection and treatment of the animals and in the removal of the tissues The following list indicates the factors to which I think attention should be given. Very likely it is not complete, but at least it is something to start with.

1. Species of animal.
2. Age of animal.
3. Previous diet.
4. Duration of the fasting period.
5. The condition of the ductless glands.
6. The season of the year.
7. Cyclic changes.
8. The state of the animal:
    a. Anaesthesia.
    b. Blood pressure.
    c. Anoxaemia.
    d. Stunning.
9. Post-mortem changes.

Even at the risk of trying your patience, I am to devote the next few minutes to a discussion of each of these factors, and since our main interest concerns the mechanism of insulin action, and there is plenty of evidence to show that there is *some* relationship between the susceptibility of the animal towards this hormone and glycogen formation, I am to pay attention in connection with each factor, in

so far as present knowledge will permit, to its influence on the normal blood-sugar level, the susceptibility of the animal to insulin and the glycogen percentages in liver and muscles.

Species of animal. I need scarcely take much time over this factor since it is well known to have an influence on all of the three functions we are studying. It is nevertheless remarkable, how unvarying the basal blood-sugar level may be in animals of widely different species; for example, it is not very different in the dog, cat, rabbit and man, but is much higher in the bird and much lower in the sheep, in the cow and possibly in other ruminants. The following average (mean) figures for observations, made mainly at the Rowett Research Institute by the Shaffer Hartman method, will serve to illustrate:

	per cent		*per cent*
Man	0.10	Rabbit	0.12
Pig	0.10	Cow	0.08
Dog	0.09	Rat	0.105
Sheep	0.07	Fowl	0.21

In each case the "basal" conditions were as much alike as possible but, of course, some of the other factors which we are to discuss may not have been completely controlled. Species may also have a marked influence on glycogen formation; for example, it is an easy matter by rich feeding with carbohydrate to cause much higher percentages, 10 and above, of glycogen to be deposited in the liver of rabbits than is possible in that of rats; and it is not unusual to find 1 per cent or more of glycogen in the muscles of the frog and dog, whereas in rats and rabbits there is seldom more than from 0.6 to 0.8 per cent. But the most striking differences are, perhaps, with regard to susceptibility to insulin: birds are highly resistant as compared with any other warm-blooded animal, and all of the latter show a much quicker response to the hormone than cold-blooded animals, for example, the frog or fish.

Age. The best example of the influence of age is in connection with the effect of insulin on glycogen formation in the liver. Goldblatt (31) showed that the amount of this substance became increased when insulin was injected into very young rabbits, whereas, as is well known, it always becomes decreased under the same conditions in adult animals. The influence of age on the other functions of carbo-

hydrate metabolism urgently awaits further investigation especially since, as Corkill (19) has shown, the rabbit is somewhat unique in showing these differences in the behaviour of glycogen.

Previous diet. Even supposing that food has been withdrawn for equal periods of time, the percentage of glycogen in the liver and possibly of the muscles seems to vary according to the nature of the preceding diet. We have noted this in several pieces of work at the Rowett Research Institute, but have never been able to define exactly what is responsible for the differences. We have not, as yet, published any of the results of our observations, but very valuable observations by Hynd and Rotter (35) bearing on the problem have appeared from the Physiological Laboratory of St. Andrews University. They fed young rats, mice and young cats for some time either on a carbohydrate-rich diet or on one of iso-caloric value consisting almost exclusively of protein and fat (cheese and butter). After fasting the animals for equal periods of time, the percentage of blood sugar was found to be decidedly higher in the animals not fed on carbohydrate than in those richly fed, although the glycogen percentage in the liver was in the reverse order, being higher in the carbohydrate group. There was little, if any difference in the glycogen content of the muscles of the two groups, but susceptibility towards injections of insulin, as judged by the rate and extent of fall of the blood-sugar percentage, was more marked in the animals not given carbohydrate. This greater susceptibility to insulin of animals deprived of carbohydrate had previously been noted by Abderhalden and Wertheimer (1) and in my laboratory by Tiitso (53). The former workers supposed that it depended on an influence on the acid base equilibrium, but I am doubtful whether this is the case, especially in the light of the more recent researches by Geiger (30).

To those of you who have to do with the treatment of diabetes in man, these effects of diet must be especially interesting. You sometimes meet with cases recalcitrant to the action of insulin. Has this anything to do with the previous diet? And again, what bearing have these observations on the changing fashions of the diabetic diet? Clinical experience may justify such changes, but what are the metabolic principles involved? It is possible that an answer to these questions would throw much light on many other problems of metab-

olism, not only of the carbohydrates but of proteins and fats as well. Hynd and Rotter (35), in the experiments to which I have just alluded, made some observations on the quantitative relationship between the fats and glycogen of the liver but nothing very definite could be demonstrated. There are innumerable problems concerning the relationship of the previous diet to carbohydrate metabolism which await investigation. Have vitamins, for example, any influence? Some years ago, Miss Jennie Macallum, at my suggestion, made a very careful study of this possibility. She divided a number of young rats into groups and fed them with diets which varied greatly in vitamin content among the different groups. She observed the blood-sugar percentage and the glycogen content of the liver and muscles of animals removed at intervals from each group, but could not show from the results that there was any definite relation between either of these and the diets. There were indeed differences as, for example, between the glycogen content of normally fed animals and those deprived of one of the growth-promoting vitamins, but it was impossible to decide whether this was not merely dependent upon variability of food intake, the activity of the animal, the occurrence of infective processes or other factors. It has, indeed, been claimed by Kinnersley and Peters (39) that vitamin *B* does affect carbohydrate metabolism; so that here again further work must be done. Then there are the mineral constituents of the diet. They certainly have some influence over carbohydrate metabolism; it may be because they are apt to affect the acid-base equilibrium of the organism or perhaps, as Geiger has supposed, because of some specific action of certain kations. I wish I had more time at my disposal to discuss these matters further, but we must pass on. I have said enough to warn those who contemplate researches in glycogen metabolism to be very cautious about the feeding of the animals they are to use.

Duration of the fasting period. From the moment absorption of food is completed, the glycogen content of the liver in a normal animal begins to decline, although it may continue for some time to increase in the muscles (*cf.* Barbour, Chaikoff, Macleod and Orr (5)). Cori has called this the post-absorptive period, during which the glycogen disappears at a rate varying in different animals, according to their size. The following results obtained by killing standard rats at later

stages of fasting will serve to illustrate how the glycogen content behaves.

Glycogen content of rats after 24 and 48 hours of fasting (Cori (17))

All values calculated per 100 grams of body weight

	LIVER	REST OF BODY	TOTAL	NUMBER OF ANIMALS
hours	*mg.*	*mg.*		
24	7±2	136±10	143±11	16
48	10±2	111±13	121±13	21
Difference.....	+3	−25	−22	

After 24 hours the liver glycogen appears to reach its lowest level: it increases in percentage thereafter, perhaps doubtfully up to the 48th hour[2] but more definitely later, as is shown in the following results obtained by Orr and Hershey (33).

	per cent
After 72 hours' starvation.....	0.21 and 0.21
After 96 hours' starvation.....	0.65, 0.23 and 0.34
After 120 hours' starvation.....	0.42, 0.23 and 0.92
After 144 hours' starvation.....	0.78, 0.78 and 0.78

This increase in the amount of liver glycogen during prolonged fasting has been shown to occur in other animals, in the dog, for example (Pflüger), and it indicates, of course, that a new formation of this substance is occurring—a gluconeogenesis. In the muscles, the glycogen content goes on declining for much longer than in the liver, but it never becomes so low as to come near the vanishing point, in a resting animal at least. But I need not occupy your time in discussing what is already so well known regarding the influence of fasting.

The condition of the ductless glands. Apart from that of the islets of Langerhans, little is known of the influence of the ductless glands and the hormones secreted by them on glycogen formation and on the other processes of carbohydrate metabolism. I cannot now go into this problem to any great extent; indeed it would require the

[2] Sahyun and Luck (50), for example, found it smaller after 48 hours than after 24. Magee and Reid (44) found it to be higher in fowls after a 96-hour than after a 24-hour fast.

time of at least one lecture even to outline it, and all we can do is to make a note, as it were, that this influence comes into play. Here, there is a vast unknown field to be explored, but the pioneers who have ventured into it have, in many instances, been so unprovided with a proper knowledge of the sources of error which attend such work that their findings do not help us greatly. There can be no doubt that the thyroid and the adrenal glands—perhaps the cortex as well as the medulla—come into play, and probably the pituitary, but it must be remembered that, even when the removal of some gland, or the injection of an extract of it, is found to affect, say, the blood-sugar level or the glycogen content of liver or muscles, this does not necessarily mean that it exercises any direct control; the changes observed may be only secondary to an influence affecting some other function. My own impression is that much more knowledge regarding the influence of species, of feeding, of fasting and of the other conditions on our list is necessary before we can hope to make much headway in this field.

The season of the year. I need not remind you of the variations which occur in the glycogen content of liver and muscles in frogs, fishes and hibernating animals according to the season of the year. Much of this has been quite clearly demonstrated by the earlier workers, for the differences are so great that they are not masked by the use of less accurate methods of investigation than we have at present. In how far season influences carbohydrate metabolism in the higher mammals is unknown, and this will be by no means an easy problem to solve, since the influence of seasonal changes on diet will have to be taken into account.

Cyclic changes. The glycogen content of the liver increases during the night and diminishes during the day independently of feeding. The first indication of these cyclic changes was obtained by Forsgren (28) who used histochemical methods; and his results have been confirmed by the use of biochemical methods by Ågren, Wilander and Jorpes (2). The changes were first observed in rabbits, but the biochemical investigations have shown that they also occur in mice and rats. It will be important to learn to what extent they occur in other animals. They occur independently of the taking of food.[3]

[3] This has more recently been denied by Higgins, Bergson and Flock, Am. J. Physiol., 1932, **102**, 673.

Even the muscles show them, though to a minor extent. It is very significant that the resistance of mice towards insulin also shows a cyclic difference, being greater in the afternoon, at the time, that is to say, when the stability of glycogen is becoming less. Ågren and his co-workers have also shown, in rabbits, that the excretion of nitrogen in the urine is greater at night, and they consider that this indicates a more rapid deamination of protein, thus furnishing, in the deaminated residues, material for the glycogen formation. I look forward to a further expansion of these investigations since if such cyclic changes occur it will be necessary to take them into account in assessing our observations on glycogen changes. Fortunately, in the routine of laboratory investigation, it has often happened that the animals have been killed at the same hour of day, but this has not always been the case.

The state of the animal. By this I mean the manner of anaesthetisation or killing of the animal prior to removal of the piece of tissue for analysis and how this sample was removed and treated. That the use of anaesthetics affects the glycogen content of the liver has been more than suspected for a long time, but it is only recently that particular attention has been given to the matter, especially in the work of Evans, Tsai and Young (26) on cats. They found that about 60 per cent of the glycogen originally present in the liver (as gauged from analysis of successive samples taken at short intervals) disappeared during a period of 60 minutes of ether anaesthesia. Probably the most rapid glycogenolysis occurs with chloroform and the least when anaesthesia is gradually induced by using amytal or some other barbiturate. The administration of anaesthetics also affects the glycogen content of the muscles, but in this case the degree of struggling must largely determine the rate at which glycogen diminishes. But I need not labour this point, for it must be self-evident.

A large number of results have been published concerning the glycogen content of both liver and muscles removed from animals after killing them by pithing or by a blow on the head. From the present point of view, the latter is probably the worst of all the methods of killing, for it first sets up serious nervous disturbances which may act both through the nerves controlling the glycogenolytic process directly and through those controlling muscular activity.

Even if one attempts by standardisation of the method to circumvent this source of error, as in researches in which the only object is to make comparisons among a group of animals, results concerning the glycogen content can be of little value, and I deplore the amount of time which I, for one, have wasted by using it. It is only within the past few years that I have come to realise the magnitude of the errors liable to be incurred by such methods, and this partly as the result of an investigation recently carried out in collaboration with I. A. Anderson (4). We started, expecting that a few weeks' work would clear up the problems in hand, but, as the results of each experiment became available, they only served to confuse us more and more, and it took many months before we were able to obtain consistent findings and to draw conclusions of any value. It may not be amiss if I give a few of the results here.

We devoted out attention more especially to the behaviour of the muscle glycogen, using a comparison of the percentages of glycogen in the two hind-legs as a criterion of the degree to which changes occurred. Closest correspondence was obtained in rats which were killed by suddenly cutting the spinal column fairly low down. Under these conditions there was often little, if any, contraction of the leg muscles and a close correspondence of glycogen on the two sides. When, on the other hand, twitching occurred, the glycogen percentages varied greatly. It may be interesting to give the actual figures (table III). Larger animals are more difficult to handle in this way but we have obtained fairly satisfactory results, as judged by comparing the glycogen percentage in the two legs, by using a guillotine built after the pattern of those once used for other purposes in France.

I have included, in the list of conditions, blood pressure and anoxaemia but I cannot say much with regard to the extent of their influence on oxygen content. These two conditions are interlocked, so to say, and the most striking example of their influence is perhaps illustrated by the rapidity with which glycogen disappears from the muscles of fishes when these are caught in trawls. When quickly netted by a landing net or even when caught on a single line and immediately brought to the surface, the glycogen content is much higher than in trawled fish even though the trawling has lasted only a few minutes. That muscular exercise is not alone responsible for

this result, we have shown by the fact that little change in glycogen content can be induced by making the fish move about actively in the water before landing them. Under these circumstances it seems to be impossible to bring about such a degree of activity that the muscles incur an "oxygen debt"; they would seem to work at a "steady state" both with regard to their oxygen requirements and their glycogen

TABLE III

Percentage of glycogen (as glucose) in leg muscles of rats killed by body transection

NUMBER	RIGHT LEG	LEFT LEG
I. Without contractions		
1	0.207	0.183
2	0.217	0.218
3	0.121	0.127
4	0.270	0.275
5	0.233	0.245
6	0.264	0.249
7	0.246	0.253
8	0.211	0.210
Average	0.221	0.220
II. With contractions		
1	0.145	0.211
2	0.205	0.137
3	0.178	0.141
4	0.143	0.177
5	0.228	0.305
6	0.103	0.081
7	0.195	0.224
Average	0.171	0.182

content. But I fear I am taking you into fields of investigation which are somewhat outside our chosen ambit. There is one further point to which I must allude, however, namely, the remarkable constancy of the glycogen content of the leg musculature in the spinal animal. Is this related to the low blood pressure of such preparations? It is not evident after decerebration alone, which would seem to support

this interpretation, but, here again, there is some confusion, since muscular activity is by no means absent after this procedure.

Post-mortem change. Finally, there is the question of post-mortem glycogenolysis. It has long been known that this process sets in early in the liver and many interesting results have been published bearing on the conditions which affect the time of its onset and the rate at which it proceeds. One fact is here worthy of note, namely, that the onset appears to occur earlier when observed by accumulation of free sugar, rather than by decrease in glycogen percentage. Purves has recently made some observations in my laboratory on this apparent difference, since, if proved to exist, it would suggest that part of the sugar is derived not directly from glycogen but from some intermediary substance, perhaps a lower dextrin, and that glycogen itself remains intact for somewhat longer. This possibility becomes all the more interesting because of the relative post-mortem stability of glycogen in the muscles. This can be judged by removing the muscles of one limb immediately after killing and those of the opposite limb at intervals thereafter. After an hour or so, no significant difference can, as a rule, be detected between the two limbs and, indeed, in some cases it is difficult to be certain that a slight increase may not have occurred. Be this as it may, there is no doubt that post-mortem glycogenolysis sets in much more slowly in the muscles than in the liver. The extent to which muscular activity, just before death, affects the post-mortem glycogenolysis is a problem which awaits further investigation.

As you are aware, a very great deal of research has been put on record regarding the influence of exercise on the percentage of glycogen in the muscles at the time of death. No useful purpose relative to the problem in hand would be served if I were to review the researches in this field, but there are two points in connection with them that I should like to emphasize. One of these is that exercise, which is not unusually severe and is, therefore, presumably performed without incurring any oxygen debt, does not cause a significant fall in the glycogen percentage of the muscles. Even when unusual activity is induced under the control of an intact nervous system, no measurable change in muscle glycogen may occur. Thus, in the Toronto laboratory, Hershey and Orr (33) compelled white rats to perform what was,

for them, unusually severe exercise, either by working on a treadmill or by making them swim for long periods of time, but no decrease in the percentage of muscle glycogen was detected. When, on the other hand, muscles are excited to abnormal activity in spinal or decerebrate preparations, by electrical stimulation of their nerve supply, or by some other purely experimental method, or when contractions are excited in dying muscle, the glycogen rapidly disappears.

The other point (to which I have already drawn your attention) is, that even a few apparently feeble twitches of a muscle or group of muscles in one extremity in a dying mammal may cause its glycogen percentage to fall considerably below that of the corresponding muscle or group of muscles of the opposite limb, which has meanwhile been kept at rest.

In the light of a knowledge of all these possible sources of error, I think it is clear that there are but few of the recorded investigations on the behaviour of glycogen after such procedures as the removal of some ductless gland or the injection of some extract or drug, or indeed *any* experimental procedure, which can be regarded as strictly accurate. Many of my own results fall under this criticism and, in the future, when such investigations must be made, I consider that the only proper method is to adopt a very strict standardisation of all the conditions of the experiment, at least until we know sufficiently well the influence of each of the factors I have discussed to be able to discount them quantitatively. Animals of the same breed and age only —litter mates, if possible—may be used; their previous feeding must be uniform, they must all be fasted for exactly the same period and used at the same time of day, handled exactly alike during anaesthetisation—there must, for example, be no emotional disturbance preceding this process—and the sampling of liver and muscle for the determination of glycogen must be performed with all the precautions which I have described.

And now let us turn back to our main problem, the mechanism of the action of insulin. What is its immediate influence on glycogen metabolism? Of the innumerable researches which have been published there are, I fear, but very few whose results can be given much weight. I will refer here to three of those summarised in table 1, in

which, I think, the possible sources of error have been more or less controlled by strict standardisation.

The first of these comprises the experiments on the spinal preparation performed by Best, Hoet and Marks (10). As I told you in my first lecture, they found that glycogen was formed in the muscles of the legs when large amounts of insulin were injected into the blood stream, usually together with injections of glucose. There can be no doubt that under these conditions glycogen becomes deposited in the muscles. The next group of experiments are those of Barbour, Chaikoff, Macleod and Orr (5). In these, white rats, of a uniform stock, housed and fed alike, were used. The ages of the animals chosen for each experiment—as judged by the body weights—were not very different, but had we then known that age is so important a factor, as Goldblatt's work on rabbits has shown it to be, we should have selected our animals with greater care in this regard. Nevertheless, we took every precaution to see that the animals injected with insulin were treated in exactly the same way as those used as controls, even to the extent of always doing the experiments at the same time of day. Notwithstanding the imperfections, I believe that the results such as shown in figure 5 are trustworthy and conclusive. The outstanding result is that there may be no increase in glycogen either in the liver or muscles after injections of insulin, sufficient in dosage to lower the blood sugar nearly to the convulsive level, or after injections of insulin given during the absorption of glucose from the alimentary canal. It was only when very large doses of insulin were injected into feeding animals that an increase in the percentage of muscle glycogen could be demonstrated, and even then it is uncertain whether the total glycogen of the body was altered, since the decrease in the total amount of liver glycogen was, as far as could be calculated, about the same as the increase in the total amount in the muscles. The results certainly do not justify us in concluding that the fall in the level of the blood sugar which follows the injection of insulin is due to conversion of some of this sugar into glycogen.

The third group of experiments to which I would refer are those of Dr. and Mrs. Cori (18), who also used the standard rat and measured the respiratory exchange of the injected animals as well as the

changes in glycogen. For the latter purpose they used the entire carcase after removal of the viscera and, in this regard, their results are more dependable than those of Barbour and others, who used only the hind limbs. Their own conclusions with regard to glycogen formation are as follows: "When the same blood sugar level was maintained in insulinized and control animals by supplying more sugar to the former than to the latter all that could be demonstrated

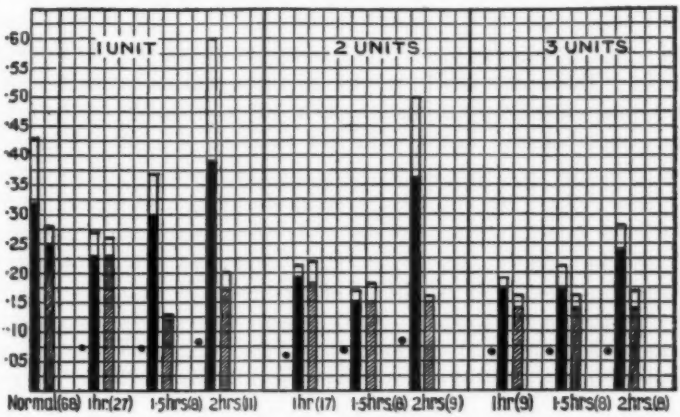

FIG. 5. INFLUENCE ON GLYCOGEN, IN WHITE RATS, OF VARYING AMOUNTS OF INSULIN INJECTED AFTER 48 HOURS' FASTING

Ordinates, per cent glycogen; abscissa, times of killing after injection; black columns, liver; cross-hatched columns, muscles. Numbers in brackets give number of animals averaged in each case, and the unshaded portion of each column, the percentile error. Black dots, blood-sugar percentage.

was a glycogen deposition of the same magnitude in both groups of rats."

To sum up the evidence offered in these three groups of researches, we may say that, although excess of insulin in the presence of a high concentration of sugar in the blood—and therefore also in the tissues—does lead to glycogen formation in the muscles, such a process cannot be responsible for the immediate or initial fall in the blood-sugar level that follows the injection of this hormone. The formation of glycogen that ultimately occurs must be secondary to other changes

and, as I said before, no evidence has so far been presented refuting the hypothesis I ventured to advance several years ago, namely, that the sugar disappearing from the blood immediately after insulin is injected, goes to form some intermediary substance of carbohydrate metabolism, which has not as yet been identified. Of course, those of you who know about the recent investigations in muscle chemistry will immediately ask the question, 'May this intermediary substance not be some form of hexose phosphate?' Perhaps. The work of the Coris in this field does not lend support to such an hypothesis, and D. J. Bell (6), in my laboratory, has confirmed the Coris' results.

I have taken so much time over glycogen metabolism that there is little to spare for a consideration of the effect of insulin on the respiratory metabolism. But I must not overlook this subject entirely. Theoretically, by observing the behaviour of the respiratory quotient, the intake of oxygen and the excretion of nitrogen, we should be able to tell the extent to which carbohydrate is being oxidised in comparison with protein and fat, and to determine from any change which might follow the injection of insulin, or of any other substance, its particular action on metabolism. But there are several possible sources of error in the interpretation of changes in the respiratory quotient, and these I have already discussed in my first lecture. Unless the CO_2-content of the blood is determined together with the respiratory quotient, conclusions drawn from changes in the behaviour of the latter cannot be regarded as final. Notwithstanding this requirement, which I may say is a very exacting one owing to experimental difficulties, I would ask you to glance for a moment at some of the recorded results concerning the effect of insulin. The curves in figure 6 show the effect on respiratory metabolism of injection into the dog was of sufficient insulin to cause hypoglycaemia accompanied by its characteristic external symptoms.

It will be observed in these experiments on dogs that there was very little change in the respiratory quotient or the O_2-consumption until the blood sugar had fallen nearly to the symptom-level, after which both of them, as well as the respiratory and pulse rates, mounted quickly. The natural interpretation is that the increasing hyperexcitability of the nervous and muscular systems was responsible for the change. That this was so became evident in further experiments

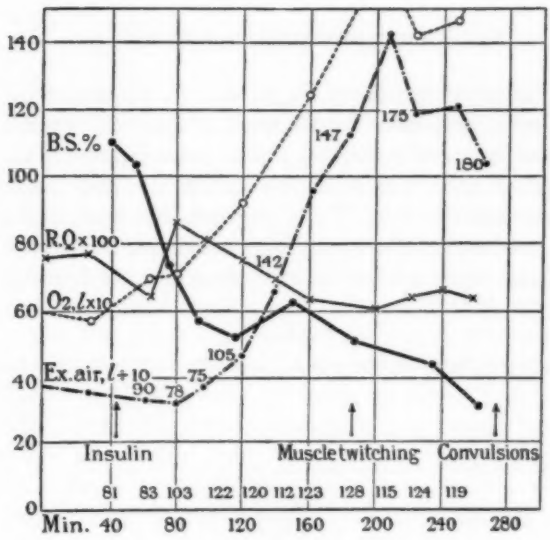

FIG. 6. CURVES SHOWING EFFECTS OF INSULIN ON BLOOD-SUGAR, R.Q., O_2 CONSUMPTION, PULSE RATE AND RESPIRATION

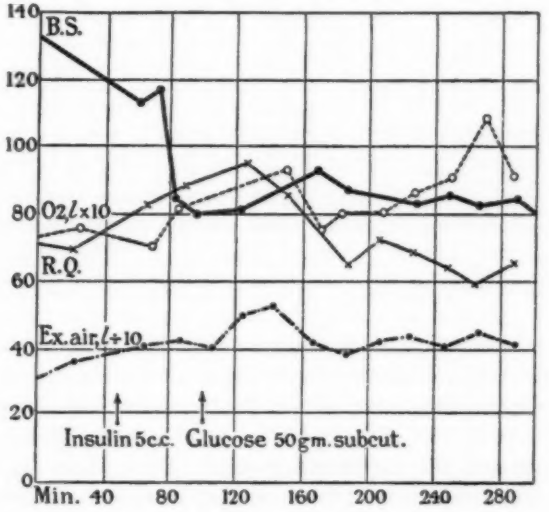

FIG. 7. CURVES SHOWING ANTIDOTING EFFECTS OF GLUCOSE ON THE RESPIRATORY CHANGES CAUSED BY INSULIN ALONE

in which the fall in the blood-sugar level was prevented by administering glucose, as is shown in the curves of figure 7. The important point is that there was no decided and constant increase in the respiratory quotient and O_2-consumption during the time, immediately following the injection, when the blood-sugar level was quickly falling. Increased combustion of carbohydrate could not therefore be given as the primary cause for the disappearance of sugar from the blood.

TABLE IV

Comparison of O_2 consumption and R.Q. in rabbits with and without insulin

	O_2	R.Q.	TOTAL CALORIES	TOTAL CARBOHYDRATE	DIFFERENCE
			In 6 hrs. per kg.		
Feeding					
	c. cm. per hr.			*g*	*g*
1. Normal	615	1.03	18.6	4.97	
Insulin	577	1.04	17.45	4.67	−0.31
2. Normal	560	1.01	16.98	4.54	
Insulin	603	1.01	19.06	5.10	+0.56
3. Normal	723	1.02	21.90	5.86	
Insulin	764	0.99	23.14	5.87	+0.01
Fasting					
1. Normal	541	0.76	15.42	0.76	
Insulin	607	0.77	17.39	1.09	+0.33
2. Normal	535	0.72	15.12	0.16	
Insulin	558	0.74	15.90	0.49	+0.33
3. Normal	720	0.72	16.90	0.22	
Insulin	657	0.78	15.77	1.06	+0.84

The results of another series of experiments are shown in table IV. These were performed on rabbits which were either feeding on carbohydrate-rich food both before and during the respiratory observations, or were subjected to a preliminary period of fasting. In the former group of animals the respiratory quotient showed that the metabolism was confined to carbohydrate, so that it would be impossible for it to be further stimulated following the injection of insulin, unless a decided rise occurred in the O_2-consumption, which, as the table

shows, did not occur. In the previously fasted animals the results were different, for insulin caused a rise in the respiratory quotient (and in O_2-consumption), so that in this case it would be correct to conclude that carbohydrate combustion had become increased, relatively to that of protein or fat or both of them. These results, then, do not show that the initial fall in the blood-sugar level following insulin injection is primarily due to an increase in carbohydrate metabolism, but I should like to make a reservation in drawing this conclusion. This depends on the fact that the removal of a small amount of sugar from the blood may cause a marked change in percentage. After all, when the percentage is 0.1, the quantity of glucose in the total volume of blood of a rabbit weighing 2 kg. is only 0.14 gm., so that only 0.07 gm. would have to be removed to bring the blood-sugar level down to the convulsive level and this amount would not produce a perceptible change in respiratory metabolism. In their observations on the rat, the Coris measured the respiratory exchange as well as the changes in glycogen. In summing up the results of their numerous observations, they state that "insulin does not possess a direct calorigenic action; *i.e.*, the increase in carbohydrate oxidation after insulin is compensated by a decrease in fat oxidation in equicaloric proportion."

In so far as normal animals are concerned, therefore, it cannot be claimed that we are at all certain as to how insulin affects respiratory metabolism. It invariably causes the respiratory quotient to rise in diabetic animals, but not necessarily in normal ones. We may therefore conclude that the fall in the blood-sugar percentage, which it induces in both, is not causally related to increased oxidation of carbohydrate. When the enemy cannot be overcome by a frontal attack he can sometimes be outflanked, and so in attacking a problem, such as our present one, progress may be made towards its solution by indirect methods. We may come to understand how insulin acts, by studying the effects of the hormone which affects carbohydrate metabolism in most respects in exactly the opposite way. This hormone is, of course, adrenaline, and I will steal a few moments to indicate what is known of its effects. To begin with, it is important to remember that the direct action of insulin is apparently restricted to carbohydrate metabolism, whereas that of adrenaline is multi-

farious, the basal metabolism, the blood circulation and many other functions being affected by it. But these different effects of adrenaline depend on dosage. The intravenous injection of adrenaline at the rate of 0.0015 mg. per kilogram of body weight per hour produces a definite increase in the blood-sugar percentage without having any effect on the basal metabolic rate or the blood pressure or any other of the numerous physiological processes upon which this hormone acts. Obviously, therefore, the problem concerning the *modus operandi* of insulin in causing hypoglycaemia is closely linked with that of the hyperglycaemic action of adrenaline and we owe to the Coris many important contributions on this subject. Perhaps the most outstanding of them all is that the rise in blood sugar is accompanied by a decrease in the glycogen of the muscles and an increase in that of the liver. Lactic acid also accumulates in the blood, in which its concentration curve runs parallel to that of the sugar. To account for these changes, the Coris suggest that adrenaline stimulates the production of lactic acid from muscle glycogen, which is then carried by the blood to the liver where it goes back into glycogen and this in turn is changed into blood sugar. When the liver, to start with, contains deposited glycogen, adrenaline accelerates its conversion into glucose, thus accounting for the hyperglycaemia. But when there is little glycogen to start with, it is obvious that the hyperglycaemia must be due to other causes, and among these it is possible that the migration of glucose from the blood to the tissues is interfered with in some way.

It is difficult to account for diminished utilisation unaccompanied by diminished oxidation unless we imagine that it is really the migration of the glucose from blood to tissue that is interfered with. It may be that this depends on accumulation in the tissues of some substance which is intermediate between glucose and glycogen and that this blocks the way, as it were, and in this connection there may be some significance in the fact that Cori has found that hexose phosphate does accumulate in the muscles following adrenaline injections.

It can be seen that this hypothesis does not involve the assumption that adrenaline really causes any extra sugar to be produced by the liver, and yet there is evidence that this hormone does have such an

effect. This has been known for years and has recently been demonstrated very forcefully in the results obtained by Markowitz (45). He injected adrenaline several times daily into fasting rabbits and found not only that the blood-sugar percentage continued to rise after each injection, but also that considerable quantities of glycogen were present in the liver and muscles when, after a week or more, the animals were finally killed. It is impossible to explain this result unless we assume carbohydrate formation from something else (gluconeogenesis). It is certainly difficult to imagine that the adrenaline has done nothing more than cause a shift of glycogen from muscles to liver and perhaps retard the migration of sugar from blood to muscles.

Is there a possibility that the primary action of insulin is to accelerate the migration of glucose from the blood either by making the glucose molecule more susceptible to metabolic change, or by increasing the avidity of the tissues for glucose, perhaps by creating a vacuum, as it were, for this substance as a consequence of its being used up in the formation of some sugar complex? We cannot tell, for all the experimental results concerning either the formation of carbohydrate compounds or the activation of glucose, by the action of insulin, are open to criticism. Some years ago, you may remember, certain biochemists announced that insulin changes the configuration of the glucose molecule, making it, they thought, more susceptible to metabolic action. Having heard of the discovery made independently by Emil Fischer and Sir James Irvine that a simple sugar is convertible into derivatives in which it exists in a less stable but probably more highly reactive form, these biochemists thought that observed discrepancies in reducing and polarising values of physiological fluids must indicate the existence in them of free gamma sugars. But all this has long since been shown to be visionary, and I would not bring the matter up here were it not that there are still some who cling to the hypothesis. Thus Thomas (52) has recently claimed that, under certain circumstances, he can detect in dialysed blood a divergence between polariscopic and reducing values, which must depend on changes in the glucose present in it, and that injection of insulin affects the results. Bell (6) has repeated, in my laboratory, some of Thomas' experiments, but is unable to agree with the latter's interpretation of his results. Bell has found that such divergences as some-

times do occur between the two values, as in blood from hyperglycaemic animals, can be very largely accounted for by the lactate present in it.

But, notwithstanding the entire absence of evidence, I have a suspicion that insulin acts primarily on the glucose molecule and that the various physiological effects which follow its injection are secondary to this change.

On many, but not all, of the processes of carbohydrate metabolism, insulin and adrenaline have antagonistic effects. The blood-sugar level and glycogen formation in the liver and muscles are thus affected, but not so the basal metabolism, or the formation of lactic acid. Of course, in view of the multifarious effects of adrenaline, it is possible that very minute adjustments in the relative amounts of the two hormones, on strictly standardised animals, may demonstrate an antagonism in the last mentioned functions, but this is unlikely. Moreover, insulin exerts effects on other processes, such as the production of the ketone bodies on which adrenaline has no effect. On account, however, of the apparent antagonism of the two hormones in their action on three of the most prominent of the processes of carbohydrate metabolism, the suggestion has been made that these processes are controlled by the relative concentrations of the two hormones in the blood.

Indeed, at one time it was even claimed that pancreatic diabetes was due to a constant preponderance of adrenaline action due to the insufficient secretion of insulin. But the experimental evidence for such a view was easily shown, especially by Stewart and Rogoff (51), to be inadequate, and at present it has very few, if any, adherents. Nevertheless, it would seem reasonable to assume that some significance must be attached to the antagonistic influence of the two hormones, at least in their final effects both on the blood-sugar level, and in certain dosages of each, on glycogen formation. Is it possible that the fine adjustment of the blood-sugar level depends on the relative concentration of the two hormones in the blood? The fact that adrenaline affects the blood-sugar level when its concentration in the blood is far below that necessary to influence the basal metabolism or the blood pressure, would fit in with such a view, but there is as

yet no evidence which directly supports it. Now that it is possible to maintain adrenalectomised animals in a fairly normal state of health, by injecting cortical extracts, it will, I think, be worth while repeating the experiments in which pancreatectomy is performed in the absence of the adrenal glands. But the recent work of Britton (11) purporting to show that cortical extract itself affects carbohydrate metabolism must be kept in mind.

If not by these two hormones, how then may the various processes concerned in the maintenance of the blood-sugar concentration be controlled? Can this control depend on some other hormone, or is it a function of the nervous system? We know that the internal secretion of adrenaline and the glycogenic function of the liver are under nerve control, and several investigators have concluded that the secretion of insulin is also so controlled. I propose to devote the next lecture to a discussion of the nerve control of the blood-sugar level but, as a preliminary to doing so, I should like to use the remaining moments of my time to-day in a rapid survey of the evidence which has been submitted in support of the view that the secretion of insulin is under nerve control.

There can be no doubt that the islets are very richly supplied with nerve fibres and that these come, at least partly, from the vagus. When this nerve is stimulated, on the left side, the blood-sugar percentage has been observed to fall, especially when the adrenal glands have been removed and the hepatic nerves cut, to prevent any counteracting effect by secretion of adrenaline or increased glycogenolysis. But the results are inconstant and unconvincing. It seemed to be a step forward when La Barre (40), instead of merely observing the behaviour of the blood sugar in the same animal, devised crossed circulation experiments in which the pancreatic vein of one animal was anastomosed with the jugular vein of a second smaller one from which the adrenal glands had been removed. He has published results which show, without exception, that when the transfusion is done while the vagus nerve is being stimulated, the blood sugar in the recipient subsequently falls. I cannot here describe the further experiments in which, by using three animals, he has shown that it is the percentage of sugar in the blood which stimulates the vagus nerve centre. All we are concerned with is whether or not the secre-

tion of insulin is under nerve control. For various reasons it has been impossible for us to repeat La Barre's experiments, but Dr. Peterson has been able to contribute some results which do not support the nerve control hypothesis. These were performed on the decerebrate cat and were of two types. In one group, the blood-sugar percentage was observed at intervals before and during stimulation of the vagus nerves, but no change was observed to occur. In another group, the extent to which the muscles of the leg reform glycogen after muscular activity was compared in vagotomised and in normal (decerebrated) animals. No difference could be detected, although de Bois (20) had previously stated that no glycogen was reformed in anaesthetised cats when the vagi were severed. This would imply not only that the presence of insulin is necessary for glycogen formation but also that it fails to be secreted in the absence of nerve control. But Dr. Peterson's results do not support either contention, for he has found that glycogen becomes reformed in fatigued muscle after pancreatectomy and, indeed, even after the other abdominal viscera have also been removed. Moreover, it has been known for years that the glycogen content of the muscles is not greatly reduced in pancreatectomised dogs. There is therefore no substantial evidence that the secretion of insulin is under nerve control.

LECTURE III

We have seen that insulin, when given to a diabetic animal, causes glycogen to be formed, raises the respiratory quotient to its normal level, and stops the over-production of sugar, but that when it is given to normal animals, these changes do not always occur and cannot be held accountable for the hypoglycaemia which invariably develops. The suggestion was also made that the control of carbohydrate metabolism in the normal animal may depend on a very accurate balance between the concentrations of insulin and adrenaline in the blood so that the very slightest disturbance in either would upset this control and produce either a fall or a rise in the blood-sugar level. But it is impossible with our present resources to investigate this possibility by direct methods, and this has led us to approach it indirectly by seeing whether changes in the rate of secretion of the two hormones brought about by stimulating the glands which produce

them might affect carbohydrate metabolism. It was hoped that by such an outflanking movement some information might be acquired which would be of some value in the main attack. Therefore, Dr. Donhoffer and I (23), a year or so ago, started experiments in continuation of those initiated by Claude Bernard who, you will remember, discovered that puncture of the floor of the fourth ventricle in rabbits led very shortly to a marked glycosuria dependent on hyperglycaemia, for which it was afterwards thought a disappearance of glycogen from the liver was responsible.

Let me draw an outline of Claude Bernard's experiment. The instrument he used was a small stylet, with a projecting spike at the end. It was pushed through the skull and, penetrating the cerebellum, entered the floor of the fourth ventricle near the nuclei of the tenth cranial nerves. The instrument was withdrawn when it was felt to strike the bony floor of the cranium so that, being pointed, comparatively little damage was done to the pyramidal tracts. Claude Bernard did not do this experiment in a merely haphazard manner. His master, Magendie, had previously shown that, although stimulation of the fifth nerve did not result in a secretion of tears, this occurred when the region of its nucleus was punctured. He himself had discovered that the liver secretes sugar—glycogen was not known in those days—and he supposed that this process might be under nerve control through the vagus nerves. He therefore stimulated these nerves electrically but, since no glycosuria occurred, he fell back on the method of puncture in the region of their centres. He had at least done something which upset the control of carbohydrate metabolism but it could not be because he had irritated the vagus centre because section of the corresponding nerves did not influence the result.

Claude Bernard's experiments have frequently been repeated, although not so much so during recent years, but little new knowledge has been gained. Donhoffer and I thought it worth while to take them up again in the light of the new knowledge about carbohydrate metabolism which has been acquired since they were originally performed. For one thing it has been shown that disturbance in carbohydrate metabolism may be set up by lesions in other regions of the brain, and this indicated that our first step should be to delimit as

narrowly as possible the centre from which results like Bernard's are obtained. We arbitrarily divided the brain of the rabbit into four regions as indicated in figure 8. The first region, anterior to the tuber cinereum; the second, between that and the anterior part of the pons, the third and fourth being the pons and medulla, respectively. It is important to bear this chart in mind and to remember that III represents the region of the pons.

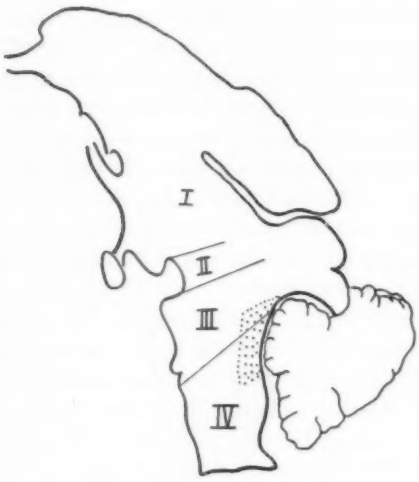

FIG. 8

In our first experiments, the animals were anaesthetised with amytal or luminal, given intraperitoneally, and we were surprised to find that puncture performed exactly as directed by Claude Bernard did not have any effect on the blood-sugar level. This was repeated many times and the absence of results surprised us all the more because I had frequently performed similar experiments using local anaesthesia and had never failed to produce hyperglycaemia. It looked as though the anaesthetic was masking the results. This led us to see whether it might be possible to cause changes in the blood-sugar level by lesions in other regions of the brain, our curiosity in this direction being aroused by recent work which is considered

to show that the chief centre of the sympathetic system is located in the subthalamic region. We argued that a lesion in this region might cause a hypersecretion of adrenaline sufficient to produce a rise in the blood-sugar level comparable with that which follows when adrenaline is injected into an animal anaesthetised with amytal. I should say here, however, that we have detected a serious flaw in this argument, for we have since found that when the dose of adrenaline is just sufficient to affect the blood-sugar level in rabbits decerebrated anterior to the pons, atropine, which seems to act like amytal in this regard, can prevent it. However, we exposed the subthalamic region and neighbouring portions of the brain, which we then subjected to various types of lesions. The results were very confusing. Occasionally the blood-sugar level would rise, but usually there was no change, and it was impossible to associate the positive results with a lesion of the subthalamic region.

It became quite evident that our outflanking movement would fail unless some method could be devised for overcoming the effect of anaesthesia on Bernard's *piqûre*. It was known that hyperglycaemia may follow this operation when it is performed on an animal in which the floor of the fourth ventricle has previously been laid bare under ether anaesthesia and this led us to find out what would happen if we were to injure the pons and adjacent regions of the brain stem during the rapidly induced and rapidly disappearing general anaesthesia which is induced by intravenous injection of amytal. As the method of injury, we chose decerebration partly because we knew that this sometimes causes hyperglycaemia and partly because the resulting preparation is one upon which further experimentation is possible without anaesthesia, since it remains alive, in the vegetative sense, for several hours after all the detectable effects of amytal have passed off. The decerebration was made through a trephine hole in the skull, in the regions indicated in the diagram. The results are shown in table V. Up to four hours after decerebration there was a slight but steady rise in the blood-sugar level after decerebration in regions I and II, but nothing comparable with that which developed when it was in region III. The results of twenty-three experiments were averaged in those figures and many more have been performed since. With occasional exceptions, determined perhaps by previous feeding

of the animals, hyperglycaemia comparable with that following injection of adrenaline has always developed. Decerebration in region IV had no similar effect.

These results gave us something to start with. They seemed to indicate that in the pons there is a centre having a profound effect on the blood-sugar level, a "diabetogenic centre," one might call it.

Before passing on to consider how this centre acts I will revert for a moment to the experiments in which decerebrations were made in regions I and II. They were particularly interesting because, as can be seen from figure 9, the decerebration often involved the subthalamic region. Nevertheless, in all those experiments with the exception of

TABLE V

Average behaviour of blood-sugar percentage after decerebration at various levels

POSITION OF DECEREBRATION	BLOOD-SUGAR PERCENTAGE						EXPERIMENTS
	Before	Immediately after	In 1 hr.	2 hrs.	3 hrs.	4 hrs.	
I	0.102	0.127	0.139	0.145	0.155	0.174	10
II	0.123	0.125	0.125	0.139	0.130	0.160	6
III	0.110	0.155	0.195	0.241	0.286	0.291	23
IV	0.109	0.137	0.123	0.135	0.131	0.126	10

that numbered 135 in the photographs, the blood sugar remained at its normal level. This, I think, shows that although it may be the case that the subthalamic region exercises some sort of control over carbohydrate metabolism, this must have little significance compared with the control exercised through the centre in the pons. You will note that some of the sections come very close to the anterior part of the pons without disturbing the sugar level, indicating that the centre is strictly limited to this region. It was important to find out if the same results would be obtained on other animals and, with this end in view, Peterson (49) conducted experiments on the cat. In four experiments the animals were decerebrated under ether anaesthesia, first of all in region II at *A*, and you will note that the blood-sugar percentage, raised by the anaesthetic, subsequently came down, so that it was often at the normal level within the hour. In no case did hyperglycaemia become developed beyond that due to the an-

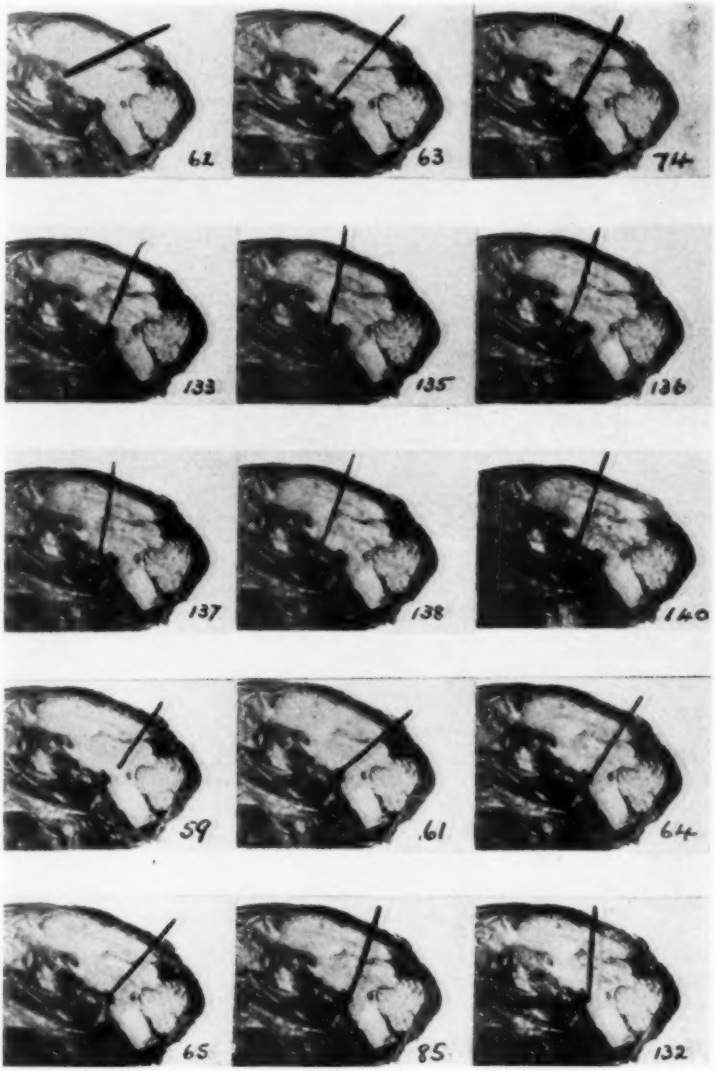

FIG. 9. PHOTOGRAPHS OF SECTIONS OF RABBIT'S BRAIN SHOWING POSITION OF DECEREBRATION

(From paper of Donhoffer and Macleod)

aesthesia. The anterior portion of the brain being entirely removed, it was now an easy matter to make a second section across the pons and the results of doing this are shown in figure 10 at *B*.

In four of the experiments the blood-sugar percentage immediately rose, the exception being an experiment in which the blood sugar still remained high from the previous anaesthetic.

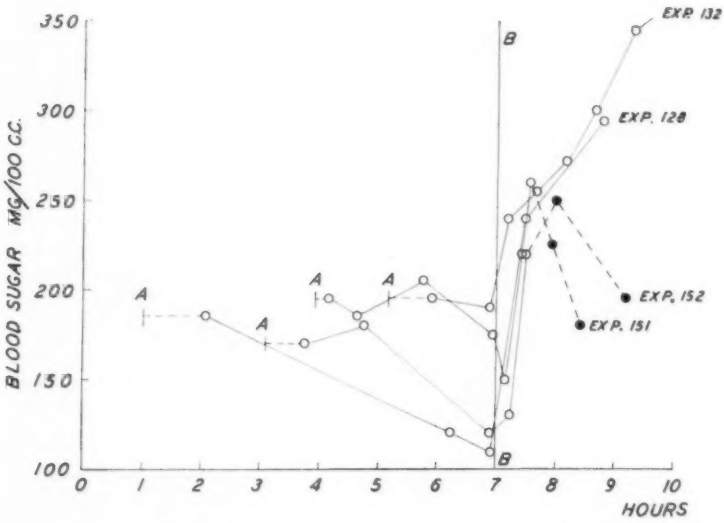

FIG. 10. At *A* in each case decerebration was performed in region II; at *B*, was performed in region III. In experiments 151 and 152 evisceration was performed and the subsequent blood sugar percentage are shown in black. (Peterson)

Then the standard white rat was tried by Magee, Bell and their collaborators (7). In Bell's experiments decerebration anterior to region III was done under ether by the string method, making two small holes on each side of the skull, threading a string down through the holes, out through the jaw, and then tying the string, a practically bloodless operation. When the effects of ether had passed off the pons was cut across with a blunt needle. The results of the first experiments performed on stock fed rats were disappointing, there being no rise in blood sugar following pontine decerebration. Later,

the procedure was modified by feeding the animals for several days before each experiment with large quantities of food especially rich in carbohydrate, with results indicated in the lower portion of the chart shown in figure 11. Under these conditions, namely, when

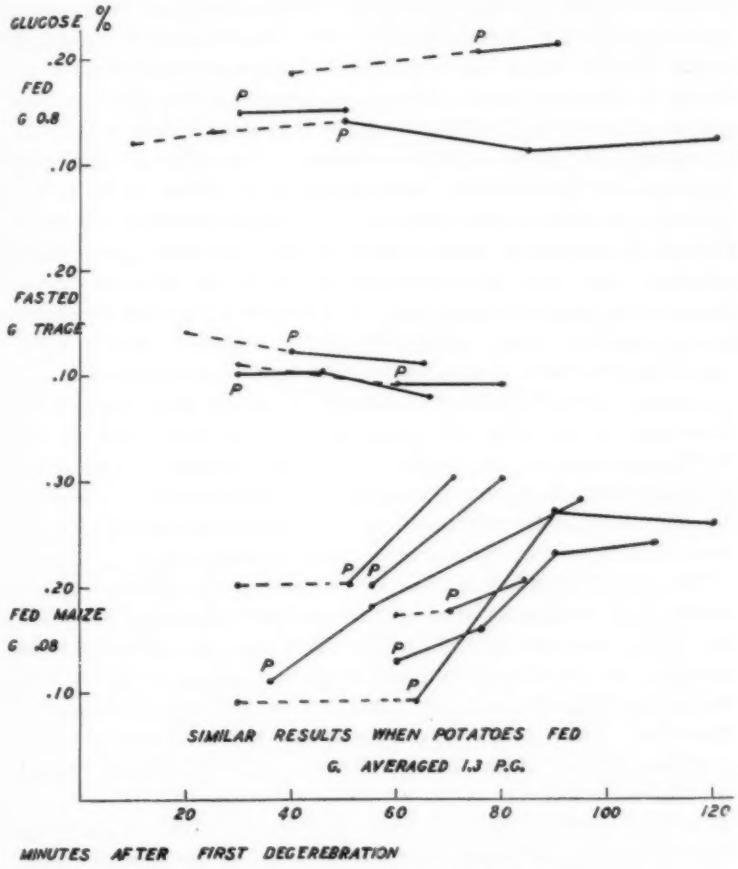

FIG. 11. EFFECT OF DECEREBRATION IN RATS

In each case decerebration was first of all performed in region II and subsequently, at *P*, in region III. Only in rats fed carbohydrate (lower set of curves) did this cause hyperglycaemia. (Magee, Bell, etc.)

large quantities of glycogen have been deposited in the liver, the lesion in the pons has the same effect in the rat as in the rabbit and the cat. Why it should prove ineffective in the fasted rat remains to be investigated.

We may now pass on to consider how the nerve centre exercises its control over carbohydrate metabolism, and the first question concerns the nerve pathway through which it acts. Since Claude Bernard had shown that the vagus was not concerned, his successors directed attention to the sympathetic pathway, and numbers of experiments were performed in which the effect of *piqûre* was studied after section of the spinal cord or the splanchnic nerves. The effect of the latter operation on decerebration hyperglycaemia is shown in table VI. Since the secretion of adrenaline from the adrenal glands is controlled through the splanchnic nerves, much attention has been given to the possibility that when an increased secretion of this hormone can no longer occur, *piqûre* is ineffective. It is supposed that this operation sets up impulses which, acting through the splanchnic nerves, bring about hypersecretion of adrenaline sufficient in itself to cause hyperglycaemia. If this could be established, it would show how metabolism may be controlled by the nervous system acting through the ductless glands, and the crucial experiment for such an hypothesis is to see whether pontine decerebration would still be followed by hyperglycaemia after adrenalectomy. We have done several experiments of this type with the results shown in table VII.

The adrenalectomy was done in two stages, the removal of the second gland being completed several days before decerebration which now failed to cause hyperglycaemia. But this does not settle the question, for an adrenalectomised animal is difficult to fatten and seldom has much glycogen in its liver, owing, perhaps, to absence of the cortex. The only way to compensate for this deficiency is to give an extract of the cortex. We can offer the results of only one experiment in which this was done, but the definite nature of the result—an increase in the blood-sugar level—justifies us I think in accepting it provisionally as evidence that the presence of the adrenal glands is not essential for the development of decerebration hyperglycaemia, a conclusion similar to that arrived at by Stewart and Rogoff (51) for the hyperglycaemia following Claude Bernard's *piqûre*.

TABLE VI

*The behaviour of the blood sugar following pontine decerebration in animals with cut splanchnic nerves**

NUMBER OF EXPERIMENT	POSITION OF DECEREBRATION	DAYS AFTER CUTTING SPLANCHNIC NERVES	BLOOD SUGAR EACH HOUR FOLLOWING DECEREBRATION				GLYCOGEN	
			1	2	3	4	Liver	Muscle
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
21	Between III and IV	1	0.16	0.15			0.91 0.00	0.34 0.32
31	III (ant. edge)	6	0.12	0.12	0.13	0.11		
35	III	3	0.16	0.15	0.15	0.10	0.84 0.01	0.25 0.28
36	III	3	0.18	0.22	0.22	0.21	0.74 0.32	0.19 0.22
53	III	3	0.13	0.13	0.13	0.12	1.22 0.73	0.61 0.52
54	III	3	0.14	0.16	0.20†		1.45 0.47	0.58
55	III‡	1	0.14	0.13	0.12	0.14	3.27 1.60	0.76 0.16

* These experiments were performed by Mr. Burns, B.Sc.

† Heart blood.

‡ Marked rigidity in muscles.

TABLE VII

Influence of adrenalectomy on the effects of pontine decerebration

Two typical experiments

NUTRITIONAL STATE OF ANIMAL	IMMEDIATELY AFTER DECEREBRATION	SUGAR (S.) AND LACTIC ACID (A.) OF BLOOD IN—				GLYCOGEN*	
		1 hr.	2 hrs.	3 hrs.	4 hrs.	Before	After
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Fed (routine)	S. 0.10 A. 0.04	0.11 0.04	0.13 0.04	0.12 0.05	0.11 0.10	M. 0.39 L. 2.02	0.40 0.15
Specially fed	S. 0.12	0.13	0.14	0.17	0.20	M. 0.64 L. 4.90	0.41 0.61

*Glycogen (per cent) in muscles (M.) and liver (L.) before and 4 hours after decerebration.

The impulses transmitted along the sympathetic pathway from the diabetogenic centre in the pons must therefore act independently of the adrenal glands, presumably by influencing the glycogenic function of the liver. This led us to investigate whether pontine decerebration would cause a change in the blood-sugar level after section of the hepatic nerves. In the rabbit, however, it is difficult to isolate all of these, as they enter the hilus of the liver without wounding branches of the portal vein, or at least interfering with the blood flowing to the liver by this vessel to such an extent that temporary disturbances in the blood-sugar level are in any case set up. We could not cut the nerves without causing such a degree of hyperglycaemia that it was futile to continue the experiments. It is quite certain, however, as R. G. Pearce and I (43) showed several years ago, that impulses controlling the discharge of sugar into the blood of the hepatic veins do of travel to the liver by the hepatic nerves for we found in the dog that the discharge increased when they were stimulated, but fell back again to the normal level when the stimulation was discontinued.

Although it is clear from these results that a mere hypersecretion of adrenaline cannot be held responsible for the hyperglycaemia following decerebration, *etc.*, it has to be remembered that the presence of the adrenal glands is important for maintaining the excitability of the sympathetic pathway. Thus, Pearce and I found that stimulation of the hepatic nerves did not lead to a rise in the sugar percentage of the blood of the hepatic veins in animals from which both adrenal glands had been removed. True it is that these older experiments were all done while the animals (dogs) were continuously under the influence of ether, which in itself disturbs the blood-sugar level, but all were treated alike, so that the conclusions, as I have stated them, are probably justifiable from the comparative results. It is possible when diabetogenic impulses are set up that one effect is that an increased secretion of adrenaline occurs and that this reinforces the other effect which is on the glycogenic function of the liver. This may account for the fact that it is less easy to induce, by decerebration or *piqûre*, a rise in the blood-sugar level in adrenalectomised animals as compared with normal ones.

In so far as it is permissible to draw conclusions from the effects of nerve section, the sympathetic to the abdomen would appear to

be at least one pathway along which the diabetogenic impulses are transmitted after pontine decerebration. This possibility made it seem worth while to see whether decerebration would affect the blood-sugar level if performed on animals injected with ergotamine, a drug which is believed to cause a block in the sympathetic pathway, but as yet I have no results to report in this connection, because of the difficulty in collecting blood[4] from the ear veins.

The conclusion that the integrity of the sympathetic nerve pathway is essential for the development of decerebration hyperglycaemia does not rule out the possibility that other influences such as changes in the internal secretion of insulin may also play a rôle. Since so many have come to believe that the internal secretion of insulin is regulated by impulses transmitted by the parasympathetic nerves to the pancreas—a question which we considered briefly at the end of the second lecture—and since it is possible, as suggested by Clark (13), that these may have an inhibitory influence, we thought it worth while to see whether pontine decerebration would cause hyperglycaemia after cutting the vagi and injecting atropine. If the decerebration stimulated the centre from which these inhibitory impulses arose, the sudden cessation of the internal secretion of insulin would cause hyperglycaemia, just as occurs after pancreatectomy, but would be ineffective after severance of the parasympathetic pathway. We actually found that pontine decerebration did not cause the blood-sugar level to rise in previously fasted rabbits to which large doses of atropine had been given after cutting the vagi, but that this occurred when the percentage of glycogen in the liver was high to start with. Under these conditions atropine had apparently no effect. These results are shown in table VIII and they would seem to show that the mechanism of sugar production controlled by the vagus differs from that controlled by the sympathetic. The former acts on a process by which sugar is produced *de novo*—a process of glyconeogenesis—and the latter on the rate of break-down of stored glycogen—a process of glycogenolysis. The small traces of glycogen

[4] Since these lectures were delivered, Miss M. Louisa Long has repeated these experiments in my laboratory by collecting the blood samples for analysis from the carotid artery. The results show that ergotamine does prevent decerebration hyperglycaemia.

which are found in the liver when only gluconeogenesis is occurring have a functional significance which is different from that of stored glycogen and whether there is also a difference in the chemical structure of the two varieties remains to be investigated. Claude Bernard was correct in speaking of the secretion of sugar by the liver.

TABLE VIII

Influence of vagotomy and atropine on behaviour of percentages of blood-sugar and glycogen of muscles (M.) and liver (L.) following pontine decerebration

Typical experiments

NORMAL	IMMEDIATELY AFTER DECEREBRATION	BLOOD-SUGAR IN—				GLYCOGEN	
		1 hr.	2 hrs.	3 hrs.	4 hrs.	Before	After
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.10	0.14	0.13	0.10	0.08		L. 0.45 M. 0.48	0.20 0.34
0.10	0.10	0.09	0.10	0.09	0.09	L. 0.19 M. 0.07	0.16 0.06
0.11	0.14	0.13	0.13	0.13	0.18	L. 2.53 M. 0.41	0.80 0.40
0.11	0.21	0.26	0.29	0.41	0.49	L. 12.20 M. 0.50	7.17 0.40
0.09	0.16	0.19	0.39	0.47		L. 16.40 M. 1.45	9.70 1.29
Averages for 10 experiments on ordinarily fed rabbits							
0.11	0.12	0.13	0.13	0.15(8)	0.17(6)	*	*

* Glycogen unknown.

These results with atropine led us to ask ourselves whether amytal might act in the same way. It is known that this hypnotic is capable of annulling the effects produced on the heart beat by vagus stimulation, and that it likewise interferes with the parasympathetic control of other functions, such as the movements of the stomach and the secretion of saliva. Unlike other hypnotics and anaesthetics amytal does not cause hyperglycaemia and the glycogen content of liver and

muscles remains unchanged in animals to which it is given. This is shown by the curves in figure 12. Is it possible, therefore, that it blocks the parasympathetic pathway much in the same way as atropine does and so prevents the secretion of sugar from the liver which ordinarily follows decerebration or the administration of other

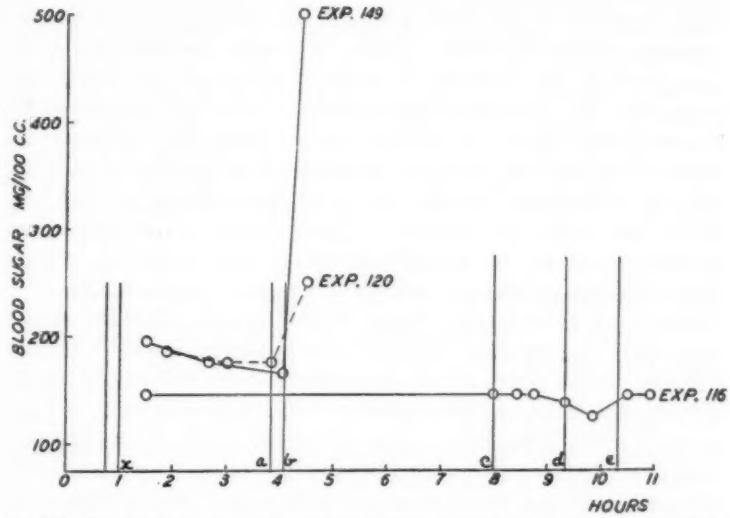

FIG. 12. EXPERIMENTS ILLUSTRATING THE DIFFICULTY OF RAISING, BY PIQÛRE, THE BLOOD-SUGAR LEVEL IN THE CAT DECEREBRATED UNDER AMYTAL

Experiment 116. Decerebration (R. 1–2) under amytal at *x*. Single puncture of pons at *c*. This puncture enlarged transversely at *d*. Fifteen gross punctures made in the pons at *e*, causing loss of blood and slight hyperpnoea.

Experiment 120. Decerebration (R. 1–2) under N_2O and O_2 at *x*. Single puncture of the pons with loss of blood at *a*.

Experiment 149. Decerebration (R. 1–2) under N_2O at *x*. Pontine decerebration (R. 3) with loss of blood at *b*.

anaesthetics? If this could be shown to be the case, it would account for the fact that only negative results were obtained when we performed Claude Bernard's *piqûre* experiment on rabbits injected intraperitoneally with sufficient amytal or luminal to induce a lasting anaesthesia although, when it was rapidly injected intravenously so

that its effect was transient, decerebration was followed by hyperglycaemia. But we must not too hastily accept this as the true explanation of the action of amytal, for this drug also causes a fall in arterial blood pressure.

To sum up, we may assume that the diabetogenic centre, acting through the parasympathetic pathway, stimulates the process of sugar secretion by the liver, perhaps indirectly by controlling the internal secretion of insulin. When this centre is stimulated, as by decerebration, the secretion of insulin is inhibited, but this cannot occur when the parasympathetic pathway is blocked by atropine, by section of the vagi or by amytal. At the same time that these inhibitory impulses pass along the parasympathetic system, stimulating ones are transmitted through the splanchnics directly to the liver where they excite the process of glycogenolysis, an accompanying increased secretion of adrenaline possibly augmenting the effect. When the splanchnics are cut, or the adrenal glands removed, or ergotamine is given in large dosage, these stimulating impulses cannot pass, hence decerebration does not cause hyperglycaemia. The chief difficulty to the acceptance of these explanations is that ergotamine should prevent hyperglycaemia from developing when there is little or no glycogen in the liver. I should indeed not be much impressed with these effects of atropine and ergotamine on decerebration hyperglycaemia were they not somewhat similar to those which follow when the drugs are given to animals during the absorption of sugar from the alimentary canal. When both drugs are given, the usual hyperglycaemia is prevented and, as recent work by Magee (7) and his co-workers at the Rowett Research Institute has shown, this cannot be entirely attributed to a delay in the emptying rate of the stomach.

And now we come to a different aspect of our problem. What is responsible for the hyperglycaemia? Does the blood show an increase in sugar percentage because more is added to it or because less is removed from it? It has been customary to believe that increased sugar production by the liver out of the glycogen stored in it is what occurs in Claude Bernard's *piqûre*, but how can this be responsible for the hyperglycaemia which occurs in the previously fasted animal? After pontine decerebration the degree of hyperglycaemia in four hours is at least as pronounced in rabbits in which there is abundant

glycogen in the liver as when it is practically non-existent, as is shown in table IX. But another possibility must be considered. There is also glycogen in the muscles. Might this be broken down to lactic acid which was then converted into glucose when it got to the liver? We have compared the percentages of glycogen in one hind limb removed before decerebration with that in the remaining one at the end of the experiment, and it is true that a decrease occurred. But, having regard to the total amount of sugar in the animal which this change in percentage would represent, is there enough to account for the hyperglycaemia? We can only answer this question by seeing how much sugar injected intravenously into a normal animal would be required to cause a degree of hyperglycaemia comparable with that of decerebration hyperglycaemia. We have done this and have

TABLE IX

Average blood-sugar percentages at hourly intervals before and after decerebration

LIVER GLYCOGEN	NORMAL	IMMEDIATE	1 HOUR	2 HOURS	3 HOURS	4 HOURS	EXPERIMENTS	GLYCOGEN
per cent								
Below 2	0.107	0.155	0.195	0.243	0.284	0.277	8	Under 1 per cent in 4 cases
Over 2	0.109	0.149	0.165*	0.218	0.280*	0.287	6	Over 5 per cent in 5 cases

* Only 5 in average.

found that the total sugar, corresponding to the glycogen loss less that corresponding to the total lactic acid accumulation, for this also occurs after pontine decerebration, is only a very small fraction of the amount of sugar necessary to cause a comparable hyperglycaemia.

It will be remembered that Claude Bernard believed that the liver secretes sugar and we imply the same thing to-day when we speak of gluconeogenesis. May it be then that decerebration causes this process to become excessive? We cannot say. If it did so, we might expect to find some by-products of the process in the blood or excreted from the body, nitrogenous compounds, for example, but these have not been found. Moreover, we should expect that a proportionately larger amount of oxygen would be absorbed by the animal, but investigations of the respiratory exchange of the decerebrated animal

have not shown that this occurs. This is shown in table X. In view of these results we are compelled to come to the conclusion that it is because of decreased utilisation of sugar by the tissues that the sugar level rises in the blood. But this cannot depend on a diminution in the rate of oxidation of the sugar, as the results in the foregoing table show. The decreased utilisation must rather be due to a retardation in the rate at which the sugar gets into the tissues, a possibility which we discussed in connection with the action of insulin in a previous lecture.

The possibility that the rate of sugar migration into the tissues is interfered with awaits further investigation. We made an attempt

TABLE X

Average consumption of O_2 and average respiratory quotient before and after pontine decerebration

	BEFORE DECEREBRATION		AFTER DECEREBRATION	
	O_2	R.Q.	O_2	R.Q.
	c.cm. per kg. an hr.		*c.cm. per kg. an hr.*	
1	420	0.73	482	0.75
2	278	0.87	373	0.82
3	291	0.78	287	0.89
4	397	0.91	347	0.91
5	390	0.95	382	0.94
6	404	0.83	423	0.86

to find a way to approach the problem by seeing whether phloridzin might have an influence on decerebration hyperglycaemia. It is well known that this drug causes glycosuria and it is supposed that it does so by lowering the threshold of the kidney for sugar. There is besides some evidence that phloridzin also lowers the sugar threshold of other tissues which suggested the possibility that it might prevent the hyperglycaemia of pontine decerebration. In a number of experiments the kidneys were tied off and in others they were left intact and the results are shown in the curves of figure 13. When the kidneys were tied *piqûre* had its usual effect, whereas, when the kidneys were left intact hyperglycaemia failed to develop, thus supplying further proof, if indeed such be necessary, that phloridzin acts specifically on

the kidney. But the results do not throw any light on our main problem.

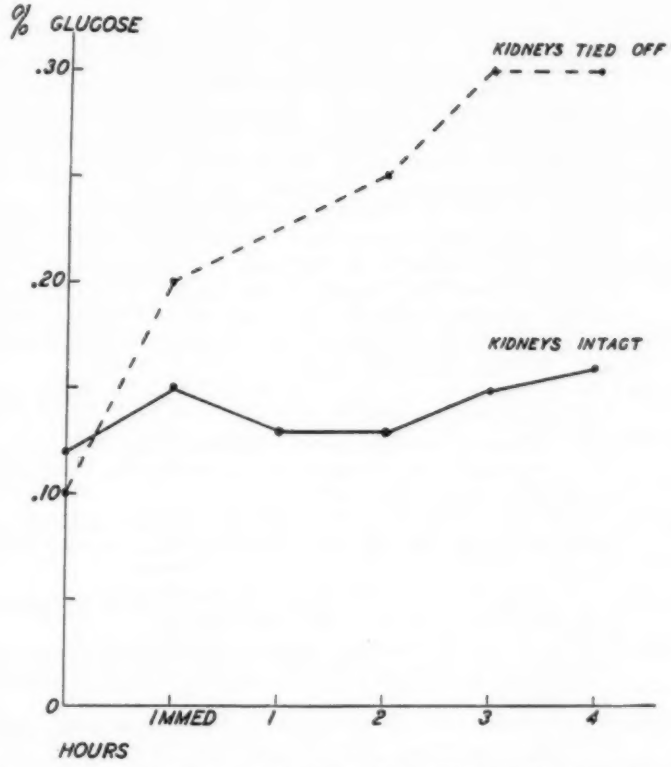

FIG. 13. CURVES SHOWING EFFECT OF PHLORIDZIN ON DECEREBRATION HYPERGLYCAEMIA

Each curve is the average of several experiments. (These experiments carried out by Miss Forster.)

I wish to take the opportunity of thanking Miss Marshall for her assistance in preparing certain of the graphs and Miss Findlay whose stenographic report of the lectures is the basis for this account of them.

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BOOK REVIEWS

Physical Chemistry for Students of Biology and Medicine. By DAVID INGERSOLL HITCHCOCK, Ph.D. 182 pp. (*Chas. C. Thomas, Springfield, Ill. & Baltimore, Md., 1932.*)

This rudimentary manual covers the elements of physical chemistry required as part of the course in physiology at the Yale Medical School of late years.

B. C.

Diagnosis and Treatment of Brain Tumors. By ERNEST SACHS. 396 pp. $10.00. (*C. V. Mosby Co., St. Louis, 1931.*)

This volume covers diagnostic problems and post-operative treatment. Designed to supplement their inadequate handling in standard textbooks, the material is collated to good purpose for the use of students.

It is difficult to accept the author's estimate that "70 to 80 per cent" of tumors can be localized by clinical study alone. One misses here an appreciative evaluation of ventriculography. Apart from the anatomical drawings, the illustrations are admirable and carefully placed so as to supplement the text.

M. B. W.

Electrosurgery. By HOWARD A. KELLY and GRANT E. WARD. 305 pp. 382 Illust. $7.00. (*W. B. Saunders Co., Philadelphia, 1932.*)

If not the first, this is the most important book which has yet appeared in electrosurgery, in which Dr. Kelly is a pioneer. Beginning with an historical introduction and a survey of the physical and experimental data, the volume covers all applications of electrosurgery, particularly in urological cases. It is well-written, profusely illustrated and provided with extensive bibliographies.

J. H. L.

The Action of the Living Cell. By DR. FENTON B. TURCK. 1933. 308 pp. $3.50. (*The Macmillan Co., New York, 1933.*)

Turck maintains that injured tissues liberate a substance of unknown composition which he calls "cytost" which produces physiological shock and contains traces of rubidium. The book sums up many years' work and incorporates the results from numerous published papers. It reveals a wide grasp of the literature dealing with experiments on the action of cytost on single cells, on animals and even on plants.

W. L.

Clinical Study and Treatment of Sick Children. By JOHN THOMSON and LEONARD FINDLAY. 5th edition. 103 pp. $3.50. (*Oxford Univ. Press, New York, 1933.*)

Findlay's revision of Thomson's classic has been thoroughly done, and will maintain its place as one of the standard texts in pediatrics.

L. E. H., JR.

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An assembly of the author's important observations on the diameters of red blood cells in health and disease. There is an interesting historical introduction.

M. W. M.

An Introduction to Practical Bacteriology. A Guide to Bacteriological Laboratory Work. By T. J. MACKIE and J. E. MCCARTNEY. 3rd edition. 451. pp. $3.50. (*Wm. Wood, New York, 1931.*)

This edition has been enlarged to about 100 pages. Designed to be a laboratory manual it shows careful and thoughtful planning, but probably will prove most useful for schools which have no provisions for technical instruction.

B. H.

Primary Syphilis in the Female. By THOMAS ANWYL DAVIES, M.D. [Lond.]. 111 pp., 17 color plates. $4.00. (*Oxford Univ. Press, New York, 1931.*)

Through the dark-field microscope, the diagnosis of primary syphilis in either sex has become a virtual laboratory procedure. As a reference book, this volume covers the clinical data, and with particularly effective plates.

J. E. M.

The History of Dermatology. By WM. ALLEN PUSEY. 223 pp. $3.00. (*C. C. Thomas, Springfield, Ill. & Baltimore, Md., 1933.*)

This, the first book on the subject in English, is highly creditable to author and publisher. The development of dermatology, from 3000 B.C. to the present time, is handled in nine attractive chapters, covering 175 pages, with a useful index of the history of different skin diseases, in alphabetic order, at the end, and highly effective illustrations. Such details as Hebra's initial contact with scabies, or the pioneer work of James C. White in our own country, will make this a very readable and handy volume for the general practitioner. There is an excellent index.

F. H. G.

Manual of Bacteriology. By ROBERT MUIR and JAMES RITCHIE. 9th ed. 866 pp. $4.75. (*Oxford Univ. Press, New York, 1932.*)

The ninth edition of this textbook is well up to the high standards set by previous ones. The chapters on the streptococci and filter-passing viruses have been

In the February issue there appears a review of "Clinical Study and Treatment of Sick Children" by John Thomson and Leonard Findlay, giving Oxford University Press, New York, as the publisher. The publisher's name should read Oliver & Boyd, London, England.

rewritten and alterations in the sections on immunity and serology bring the material up to date.

B. H.

Inherited Abnormalities of the Skin. By E. A. COCKAYE. 394 pp. $8.00. (*Oxford Univ. Press, New York, 1933.*)

Important to the dermatologist, and to those interested in genetics, this volume stresses the rôle of heredity in the classification of cutaneous disorders. A veritable "mine of information," it should stimulate more exhaustive records of cutaneous abnormalities discoverable in routine physical examinations.

The introductory chapter covers such phases as physiological abnormalities, metabolic errors, developmental errors in elastic tissue, dyskeratoses, ectodermal dystrophies, disorders of growth. Photographs of those rare conditions were precluded by the mere size of the book, but a useful bibliography is attached to each topic.

The author deserves credit for an invaluable reference book which is also an acceptable contribution to medical literature.

I. R. P.

Obstetrical Nursing. By CAROLYN CONANT VAN BLARCOM. 3rd edition. 251 illust. 12 charts. 651 pp. $3.00. (*Macmillan Co., New York, 1933.*)

New chapters on mental hygiene for the prospective mother and during the nursing period, as well as interesting illustrations, have been added to this authoritative work.

M. G. B.

Peptic Ulcer. [Annals of Roentgenology, Vol. X.] By JACOB BUCKSTEIN. Introduction by Harold E. Santee. 338 pp. $12.00. (*Paul B. Hoeber, Inc., New York, 1930.*)

A valuable contribution to a sphere of Roentgen diagnosis which has rapidly expanded in the past few years.

W. B. F.

Cutaneous X-ray and Radium Therapy By HENRI H. HAZEN. 166 pp. Illust. $3.00. (*C. V. Mosby Co., St. Louis, 1931.*)

Presents the fundamental aspects of cutaneous radiation therapy at a glance.

W. B. F.

Allergy and Applied Immunology By WARREN T. VAUGHAN, M.D. 359 pp. Illust. (*C. V. Mosby Co., St. Louis, 1931.*)

This publication covers allergy and applied immunology in a very satisfactory manner. The information conveyed will be of help to the physician who is constantly encountering allergic problems in his diagnostic practice. However, it seems a mistake to publish "a handbook for physician and patient." Many of the terms in the volume, while understandable to a doctor, are quite beyond

the medical knowledge of the average layman. Many new and unnecessary terms are coined by the author, which he defines in detail and illustrates by citing examples. These terms, which are rarely seen in the literature will undoubtedly mean little to a layman. The bibliography is quite complete.

L. N. G.

Paralysis in Children. By R. G. GORDON and M. FORRESTER BROWN. Illust. 328 pp. $4.00. (*Oxford Univ. Press, New York, 1933.*)

This book of some 300 pages is apparently intended to aid the orthopedic surgeon in the understanding of neurological conditions of childhood which he is so often called upon to treat and about which he is so often inadequately informed. Conversely, it might be of value to the neurologist who rarely has any real understanding of the principles of orthopedic work Its purpose is therefore laudable. A great deal is compressed within a small number of pages. In the introductory section, there are discussions of the physiology of movement, the common causes of paralysis and the diagnostic significance of physical signs. Some anatomical charts are given, but they are not always well chosen. In the second part, there are brief discussions of the commoner diseases of the nervous system with a few references and, in the final section, various methods of orthopedic treatment are described.

F. R. F.

BOOKS RECEIVED. WELCH MEDICAL LIBRARY

Non-Tropical Sprue. [*A Study in Idiopathic Steatorrhoea*]. By TH. E. HESS THAYSEN. 1932. 258 pp. Oxford Univ. Press, N. Y.

Report to the U. S. Government on Tuberculosis. With some Therapeutic and Prophylactic Suggestions. By S. ADOLPHUS KNOPF. 1933. 59 pp. National Tuberculosis Assoc., N. Y. $1.15.

The Technique of Local Anesthesia. By ARTHUR HERTZLER. 1933. 5th edit. Illust. 292 pp. C. V. Mosby Co., St. Louis. $5.00.

The Physiological Effects of Menstruation. By MARY CHADWICK. 1932. 70 pp. Nervous & Mental Disease Publ. Co., N. Y.

La Diathermie et ses Applications Médicales. 1933. Deuxième Edition. 133 pp. [unbound]. [1. Electrothérapie]. Gauthier-Villars, Paris, 20 frs.

Posture. Its Relation to Health. By FRANK D. DICKSON. 1932. 118 illust. 213 pp. J. B. Lippincott Co., Phila. $5.00.

Bioassays. A Handbook of Quantitative Pharmacology. By JAMES C. MUNCH. 1931. 958 pp. Williams & Wilkins Co., Baltimore, Md. $10.00.

Diseases of the Coronary Arteries. [*Myocarditis*]. 1932. Illust. 164 pp. C. V. Mosby Co., St. Louis. $5.00.

Foods In Health and Disease. By LULU G. GRAVES. 1932. 390 pp. Macmillan Co., N. Y. $3.50.

Diet in Sinus Infections and Colds. [*Recipes and Menus by Elza Mes*]. By EGON V. ULLMANN. 1933. 166 pp. Macmillan Co., New York. $2.00.

A Study of Hairs and Wools, Belonging to the mammalian group of animals, including a special study of Human Hair. By JOHN GLAISTER. Cairo, Misr Press, Egyptian University, Faculty of Medicine, Publication no. 2. 1931. 187 pp.

Dangerous Drugs. By ARTHUR WOODS. 1931. Yale Univ. Press, New Haven, Conn. 123 pp. $2.00.

Handbook of Vaccine Treatment of Chronic Rheumatic Diseases. By H. WARREN CROWE. 1932. 2nd edit. 79 pp. Oxford Univ. Press., N. Y. $.80.

Sleep. By R. D. GILLESPIE. 1930. 267 pp. Wm. Wood & Co., N. Y. $3.25.

The Conquest of Old Age. Methods to Effect Rejuvenation and to Increase Functional Activity. [Translated by Eden & Cedar Paul]. 1931. 307 pp. E. P. Dutton & Co., N. Y. $5.00.

The Medical Secretary. By MINNIE G. MORSE. 1933. 162 pp. Macmillan Co., N. Y. $1.50.

Your Teeth and Their Care. A Simple Explanation of Dentistry, Dental Procedures, and The Relation of the Dentist to the Public. 1932. Illust. By Car. W. Adams. 141 pp. C. V. Mosby Co., N. Y. $1.25.

Diabetes. Its Control by the Individual and the State. [Harvard Health Talks]. By ELLIOTT P. JOSLIN. 1931. 70 pp. Harvard Univ. Press, Cambridge, Mass. $1.00.

Mayo Clinic. Collected Papers. Rochester, Minn. Collected Papers of the Mayo Clinic & Mayo Foundation. 1929. W. B. Saunders, Phila., $13.00.

Diseases of Women. By FRANKLIN I. SHROYER. 1929. 103 pp. Richard G. Badger, Boston.

Trauma, Disease Compensation. By A. J. FRASER. 1930. 524 pp. F. A. Davis Co., Phila. $6.50.

Injuries and Sport. A General Guide for the Practitioner. 1931. 543 pp. Oxford Univ. Press, N. Y. $8.00.

Surgical Operations. By E. W. HEY GROVES. 1933. 3rd Edition. Illust. Oxford Univ. Press., N. Y. $4.50.

Fractures of the Jaws. By ROBERT H. IVY and CURTIS, LAWRENCE. 1931. 180 pp.

Varicose Veins and Haemorrhoids, And Their Treatment. [With a Preface By Aug. Krogh], 1932. [Unbound]. 25 figures & 3 colored plates. 149 pp.

Food In Health and Disease. By LULU G. GRAVES. 1932. 390 pp. Macmillan Co., N. Y. $3.50.